

## Neuroimmune crosstalk in the cornea

Wu, Mengliang; Hill, Lisa J.; Downie, Laura E.; Chinnery, Holly R.

DOI:

[10.1016/j.preteyeres.2022.101105](https://doi.org/10.1016/j.preteyeres.2022.101105)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Wu, M, Hill, LJ, Downie, LE & Chinnery, HR 2022, 'Neuroimmune crosstalk in the cornea: The role of immune cells in corneal nerve maintenance during homeostasis and inflammation', *Progress in Retinal and Eye Research*. <https://doi.org/10.1016/j.preteyeres.2022.101105>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# Neuroimmune crosstalk in the cornea: the role of immune cells in corneal nerve maintenance during homeostasis and inflammation

## Contents

|  |    |
|--|----|
| 1. Introduction.....   | 1  |
| 2. Assessing corneal nerves and immune cells .....                                 | 3  |
| 2.1. In vivo confocal microscopy.....  | 3  |
| 2.2. Animal models .....   | 5  |
| 3. Corneal innervation.....  | 6  |
| 3.1. Corneal innervation anatomy and function.....                                 | 6  |
| 3.2. Changes to corneal sensory innervation in ocular conditions.....              | 10 |
| 3.3. Changes to corneal sensory innervation in systemic conditions.....            | 18 |
| 4. Corneal immune cells .....  | 22 |
| 4.1. Dendritic cells (DCs) .....   | 23 |
| 4.2. Macrophages .....   | 25 |
| 4.3. Contribution of infiltrating immune cells to corneal nerve regeneration ..... | 26 |
| 5. Corneal neuroimmune interactions .....  | 27 |
| 5.1. Interactions between corneal nerves and intraepithelial immune cells.....     | 28 |
| 5.2. Interactions between corneal nerves and macrophages .....                     | 37 |
| 5.3. Interactions between corneal nerves and other infiltrating immune cells.....  | 39 |
| 5.4. Corneal neuroimmune interactions in ocular pain .....                         | 41 |
| 5.5. Role of aging in corneal neuroimmune interactions .....                       | 42 |
| 5.6. Autonomic nerves in corneal neuroimmune interactions .....                    | 43 |
| 5.7. Summary of corneal neuroimmune interactions .....                             | 43 |
| 6. Recent advances in promoting corneal nerve regeneration .....                   | 53 |
| 6.1. Neurotrophic/growth factors .....   | 53 |
| 6.2. Anti-fibrotics.....   | 56 |
| 6.3. Autologous serum and platelet-rich plasma eye drops .....                     | 56 |
| 6.4. Omega-3 fatty acid supplementation .....                                      | 57 |
| 7. Future directions and perspectives .....  | 58 |
| References.....  | 64 |

## Abbreviations

---

|       |   |              |   |
|-------|---|--------------|---|
| ACAID | Anterior Chamber-Associated Immune Deviation        | MHC II       | class II major histocompatibility complex         |
| APCs  | antigen-presenting cells                            | MMC          | mitomycin C                                       |
| CCR2  | C-C chemokine receptor type 2                       | NGF          | nerve growth factor                               |
| CGRP  | calcitonin gene-related peptide                     | NK1R         | neurokinin-1 receptor                             |
| CNS   | central nervous system                              | NPY          | neuropeptide Y                                    |
| CNTF  | ciliary neurotrophic factor                         | PEDF         | pigment epithelial-derived factor                 |
| CXL   | corneal collagen cross-linking                      | PK           | penetrating keratoplasty                          |
| DCs   | dendritic cells                                     | POAG         | primary open-angle glaucoma                       |
| DED   | dry eye disease                                     | PRK          | photorefractive keratectomy                       |
| DHA   | docosahexaenoic acid                                | SBNP         | sub-basal nerve plexus                            |
| HIV   | glial cell-derived neurotrophic factor              | SMILE        | small incision lenticule extraction               |
| HSV   | herpes simplex virus                                | SNT          | superficial nerve terminal                        |
| HZV   | herpes zoster virus                                 | SP           | substance P                                       |
| IGF   | insulin-like growth factor                          | TGF- $\beta$ | transforming growth factor beta                   |
| IL    | interleukin   | Treg         | regulatory T cell                                 |
| IVCM  | <i>in vivo</i> confocal microscopy                  | TRPA1        | transient receptor potential ankyrin 1            |
| LASEK | laser epithelial keratomileusis                     | TRPM8        | transient receptor potential melastatin 8         |
| LASIK | laser-assisted <i>in situ</i> keratomileusis        | TRPV1        | transient receptor potential vanilloid receptor 1 |
| MANF  | mesencephalic astrocyte-derived neurotrophic factor | VEGF         | vascular endothelial growth factor                |
| MDSCs | myeloid-derived suppressor cells                    | VIP          | vasoactive intestinal peptide                     |

---

### 1 *1. Introduction*

2 The cornea is one of the most densely innervated tissues in the human body, with a nerve  
3 fiber density of approximately 46 mm/mm<sup>2</sup> at the basal epithelium layer near the corneal  
4 apex (Marfurt et al., 2010). Corneal nerves detect endogenous and exogenous signals to  
5 maintain the homeostasis of the ocular surface by regulating tear production, modulating  
6 blink reflexes, and releasing numerous neurotropic substances (Beuerman and  
7 Schimmelpfennig, 1980; Nishida, 2005). The corneal sensory nerves are supplied by the  
8 ophthalmic branch of the trigeminal nerve. In the epithelium, they stratify to form the so-  
9 called sub-basal nerve plexus (SBNP), also termed the intraepithelial corneal basal nerves  
10 (Stepp et al., 2020), at the level of the basal epithelium. The superficial nerve terminals  
11 (SNT) arising from the SBNP terminate as free nerve endings within the apical layers of the  
12 epithelium (Figure 1).

13

14 Although the cornea lacks blood vessels and is regarded as an immune privileged tissue, there  
15 are distinct populations of resident immune cells distributed throughout the corneal  
16 epithelium and stroma, including dendritic cells, macrophages, mast cells and innate  
17 lymphoid cells (Liu and Li, 2021). In addition to these resident immune cells, inflammatory  
18 cells such as neutrophils and adaptive T cells can infiltrate into the cornea to participate in the  
19 pathology of many corneal diseases (Bron et al., 2017; Yu et al., 2022).

20

21 Neuroimmune interactions, relating to the physical and functional interplay between nerves  
22 and immune cells, have been investigated in many tissues, including the respiratory  
23 epithelium (Veres et al., 2009), meninges (Schain et al., 2018), skin (Kashem et al., 2015)  
24 and cornea (Hamrah et al., 2016; Jiao et al., 2020a). Many molecular components have been  
25 co-identified in the immune and nervous systems. For example, receptors for  
26 neurotransmitters that are found on nerves, such as acetylcholine receptors, are also expressed  
27 on DCs, macrophages and T cells, suggesting a role for neural modulation of immune  
28 responses (Kawashima et al., 2015; Wang et al., 2003). Recent studies have shown that ‘anti-  
29 inflammatory’ M2 macrophages, DCs and adaptive immune cells (T cells and B cells) play a  
30 pivotal role in the mechanisms involved with peripheral nerve degeneration and regeneration  
31 (Chen et al., 2015). With wider use of *in vivo* confocal microscopy (IVCM) in clinical and  
32 research settings, and the development of transgenic animal models, considerable attention  
33 has been given to understanding the interaction between corneal nerves and immune cells  
34 (Bitirgen et al., 2018a; Choi et al., 2017; Gao et al., 2016a).

35

36 Neurogenic inflammation in the cornea provides evidence for corneal neuroimmune  
37 crosstalk. Corneal nerve damage induces immune responses to promote the clearance of  
38 disrupted axons, and release of neurotrophic factors that trigger inflammation (Lasagni Vitar  
39 et al., 2022). The inflammatory response, including activation of immune cells and released  
40 cytokines, can further induce nerve damage, to propagate the corneal neuroinflammation  
41 (Launay et al., 2016). The reciprocal relationship between immune cells and sensory nerves  
42 complicates the picture of neuroimmune interactions in the cornea. Many studies have  
43 demonstrated a negative association between corneal innervation and activated immune cells  
44 (D'Onofrio et al., 2021; Xu et al., 2021). However, there are also redress regulations in the  
45 course of neurogenic inflammation and neuroinflammation, i.e., some immune cell

46 phenotypes, cytokines and neurotrophic factors may contribute to protecting corneal  
47 innervation (Gao et al., 2016a; Liu et al., 2018). Understanding the mechanisms underlying  
48 corneal neuroimmune crosstalk is important for developing new therapeutic strategies for  
49 corneal healing and nerve regeneration.

50

51 This review summarizes and compares findings from studies that have investigated  
52 neuroimmune crosstalk (i.e., the reciprocal interaction between immune cells and sensory  
53 nerves) in the cornea, with the goal of reframing current dogma that corneal immune cells are  
54 negatively associated with corneal nerve integrity. Clinical trial investigations of emerging  
55 therapies, and novel treatment approaches currently in pre-clinical development, for corneal  
56 nerve regeneration associated with immunomodulation, are also described.

57

## 58 *2. Assessing corneal nerves and immune cells*

### 59 *2.1. In vivo confocal microscopy*

60 *In vivo* confocal microscopy (IVCM), a non-invasive real-time imaging technique, has  
61 advanced the evaluation of the corneal architecture in humans (De Silva et al., 2017; Niederer  
62 and McGhee, 2010). IVCM provides high-resolution, *en face* images to enable qualitative  
63 and quantitative analyses of corneal structures, including nerves, epithelial cells and immune  
64 cells (Figure 2). Corneal SBNP characteristics have been clinically investigated in health and  
65 disease using IVCM (Chinnery et al., 2021a; Cruzat et al., 2017; Malik et al., 2003;  
66 Petropoulos et al., 2020). Moreover, IVCM has been useful to identify and assess corneal  
67 immune cell characteristics, such as their density, distribution and morphology, and, more  
68 recently, their association with corneal nerves, in a wide range of ocular and systemic  
69 diseases. Studies have reported corneal immune cell changes using IVCM in conditions such  
70 as dry eye disease (Aggarwal et al., 2020), infectious keratitis (Kwon et al., 2018) and  
71 pterygium (Wang et al., 2010), as well as systemic neurodegenerative diseases that are not  
72 typically known to impart clinically obvious ocular surface changes, such as multiple  
73 sclerosis (Bitirgen et al., 2017) and mild cognitive impairment (Dehghani et al., 2020). There  
74 are also an increasing number of studies reporting negative correlations between corneal  
75 SBNP density and intraepithelial immune cell density (Cavalcanti et al., 2018; Cruzat et al.,  
76 2011), suggesting that corneal immune cells may be involved with, or respond to, corneal  
77 neuropathy.

78

79 Although IVCN is a valuable clinical tool for assessing corneal sensory nerves and immune  
80 cells, it has some limitations. The visibility of corneal features within the image field can be  
81 affected by various factors including image quality, imaging depth and post-capture image  
82 enhancements, potentially leading to inconsistencies in the delineation of the sub-basal nerve  
83 plexus and subsequent nerve parameter measurements (Patel and McGhee, 2013). In view of  
84 these considerations, a tool was developed to assess the methodological quality of clinical  
85 studies using laser-scanning IVCN when evaluating corneal nerve parameters (De Silva et  
86 al., 2017). The tool can also be prospectively used by researchers when designing IVCN  
87 studies, to minimise potential biases when quantifying corneal parameters, such as nerve and  
88 immune cell densities.

89

90 A practical consideration with IVCN is that it can only be used to visualize nerves  
91 comprising the SBNP, but rarely the SNT; this latter nerve plexus plays an important role in  
92 corneal sensory function, particularly as related to the sensitization of nociceptors. In  
93 addition, although the dendriform cells observed using IVCN at the basal epithelium are  
94 believed to be CD11c<sup>+</sup> dendritic cells (DCs), based on *ex vivo* immunostaining data of human  
95 corneal flatmounts (Yamagami et al., 2005; Zhivov et al., 2005), their specific surface  
96 markers cannot be verified *in vivo*. Currently, phenotypic classification of *in vivo* corneal  
97 epithelial immune cells relies mostly on morphological characteristics, with immune cells  
98 without dendrites considered ‘immature’ DCs, and those with elongated cell processes  
99 referred to as ‘mature’ DCs (Chinnery et al., 2021b). Evidence for the existence of  
100 “immature” DCs without dendrites in the healthy corneal epithelium of rodents is lacking;  
101 this could be explained by inter-species differences, or, more controversially, may indicate  
102 that DCs without dendrites in human corneas represent a different cell population altogether  
103 (Loi et al., 2022). For this reason, when referring to immune cells in the human corneal  
104 epithelium more broadly, we will refer to them as “immune cells”, except when describing  
105 findings from clinical studies where the authors have used the term “dendritic cells” (or  
106 “DCs”).

107

108 2.2. *Animal models*

109 Animal models offer the opportunity to garner more detailed knowledge about the  
110 morphology, distribution and phenotype of corneal nerve structures and immune cell  
111 populations, owing to the capacity to perform *ex vivo* immunostaining and high-resolution  
112 confocal microscopy. This is especially critical when evaluating the apically-projecting SNT  
113 that cannot be consistently imaged using current clinical IVCM devices. Animal studies can  
114 also be used to determine the identity of corneal immune cell and nerve subpopulations, and  
115 to investigate the molecular mechanisms underlying corneal nerve repair.

116

117 Transgenic mouse models that allow for direct visualization of peripheral nerves have been  
118 reviewed recently (Yamakawa et al., 2020). The *thy1*-YFP transgenic mouse, a  
119 neurofluorescent murine model whereby the *thy1* gene promoter expresses yellow fluorescent  
120 protein, can be used to readily visualize corneal sensory nerves *in vivo*, although only  
121 approximately 46% of corneal nerves express the reporter protein (Yu and Rosenblatt, 2007)  
122 (Figure 3A). The transgenic Gaba<sub>B1</sub>-GFP reporter mouse, expressing fluorescent G-protein  
123 coupled receptors for the neurotransmitter  $\gamma$ -aminobutyric acid, has been validated for corneal  
124 nerve visualization by labeling about 90% of TuJ1<sup>+</sup> (class III beta-tubulin) corneal nerve  
125 fibers (Hanack et al., 2015). The TRPM8-eYFP transgenic mouse that expresses fluorescent  
126 Transient receptor potential melastatin 8 (TRPM8) also provides a means for observing  
127 corneal cold thermoreceptor fibers (Parra et al., 2010).

128

129 In terms of transgenic models used to visualize other corneal features, such as immune cells,  
130 corneal DCs can be readily identified in CD11c reporter mice using intravital microscopy  
131 (Lee et al., 2010; Seyed-Razavi et al., 2019); when crossed with *thy1*-YFP mice, nerves, DCs  
132 and their physical interactions can be examined using intravital multiphoton microscopy (Fig  
133 3B) (Jamali et al., 2020b). Transgenic reporter Cx3cr1 GFP ‘knock-in’ mice can also be used  
134 to visualize resident corneal epithelial Cx3cr1<sup>+</sup> DCs (Chinnery et al., 2008; Chinnery et al.,  
135 2015; Chinnery et al., 2007). Heterozygous Cx3cr1<sup>GFP/+</sup> mice have a targeted replacement of  
136 the CX3CR1 gene by GFP, enabling visualization of Cx3cr1<sup>+</sup> cells, which are resident to the  
137 mouse corneal stroma and epithelium (Fig 3C). Cx3cr1<sup>GFP/GFP</sup> (homozygous) mice also  
138 express GFP, but are functionally Cx3cr1-deficient. Interrogation of the function of Cx3cr1  
139 signaling in the mouse cornea revealed that Cx3cr1-homozygous mice, lack resident

140 epithelial DCs in the healthy cornea (Fig 3D-F) (Chinnery 2007). In these mice, stromal  
141 macrophages appear phenotypically normal, but there is a striking loss of intraepithelial DCs  
142 (Fig 3E and F). Other transgenic mouse models, such as CD11c and CD207-diphtheria toxin  
143 mice, in which corneal DCs can be pharmacologically depleted either systemically or via  
144 subconjunctival injection, also provide evidence for the possible neuroprotective function of  
145 DCs during corneal homeostasis and wound healing (Choi et al., 2017; Gao et al., 2016a; Gao  
146 et al., 2011).

147

### 148 *3. Corneal innervation*

#### 149 *3.1. Corneal innervation anatomy and function*

150 The outermost layer of the cornea consists of a stratified squamous, non-keratinized  
151 epithelium, innervated by a rich sensory nerve supply (Figure 1). Corneal nerves originate  
152 from the ophthalmic division of the trigeminal ganglion (cranial nerve V), travelling  
153 suprachoroidally and ultimately branching to form the limbal plexus where they penetrate the  
154 corneoscleral limbus (Al-Aqaba et al., 2010; Marfurt et al., 1989). The limbal plexus  
155 branches and enters the cornea to form the stromal nerve trunks before turning anteriorly  
156 towards the ocular surface (Muller et al., 2003). Nerve branches arising from the anterior  
157 stromal plexus divide further and run parallel between the basal epithelium and Bowman's  
158 layer, forming the so-called SBNP. The plexus of SBNP axons typically forms a distinctive  
159 whorl-like pattern approximately 2.5 mm infero-nasal to the corneal apex (Marfurt et al.,  
160 2010) (Figure 4A). Several nerve branches of the SBNP turn upward, penetrating vertically  
161 through the epithelium, and terminating just beneath the epithelial surface as the SNT (Muller  
162 et al., 1996) (Figure 4B). Using block-face scanning electron microscopy, the SBNP has  
163 recently been shown to have physical and functional interactions with the basal corneal  
164 epithelial cells (Courson et al., 2019; Parlanti et al., 2020). The SNT create large nociceptive  
165 fields, with an estimated density of about 605 nociceptive terminals per square millimeter in  
166 the central cornea of humans that can rapidly respond to mechanical, thermal and chemical  
167 stimuli (Marfurt et al., 2010).

168

169 Sensory nerves in the human cornea are either nociceptive A $\delta$  or C-type fibers. The A $\delta$  fibers  
170 are myelinated and have a fast conducting velocity and a relatively large diameter (1 to 5  
171  $\mu$ m), whereas the slow-conducting C fibers are unmyelinated and thinner in diameter (0.2 to



172 1.5  $\mu\text{m}$ ) (Al-Aqaba et al., 2019). Approximately 70% of corneal sensory fibers are of the  
173 polymodal subtype, most of which are unmyelinated C type nerves. Polymodal nociceptors  
174 convey sharp and sustained pain through slow-conducting C fiber activation in response to  
175 mechanical, heat and chemical stimuli, with a sustained discharge of nerve impulses (Downie  
176 et al., 2021). Polymodal nociceptors can also be activated by endogenous stimuli and  
177 inflammatory mediators (Alamri et al., 2015; MacIver and Tanelian, 1993). In addition to the  
178 sensation of pain, polymodal nociceptors contribute to reflex tear secretion caused by corneal  
179 stimulation (Acosta et al., 2004). The transient receptor potential vanilloid receptor 1  
180 (TRPV1) plays a significant role in nociceptive transduction in polymodal receptors. It has  
181 been shown that over 90% of TRPV1<sup>+</sup> corneal afferent neurons are likely to be polymodal  
182 nociceptors in guinea pigs (Alamri et al., 2015), and TRPV1 is also expressed in  
183 intraepithelial nerve terminals in the mouse corneal epithelium (Jiao et al., 2021). Transient  
184 receptor potential ankyrin 1 (TRPA1) and acid-sensing ion channels are also involved in pain  
185 sensations transmitted by polymodal nociceptors (Bandell et al., 2004; Callejo et al., 2015).  
186 The activities of TRPV1 and TRPA1 are modulated by inflammation, which is proposed to  
187 underpin ocular discomfort in inflammatory conditions, such as allergic keratoconjunctivitis  
188 (Acosta et al., 2013).

189

190 Another 20% of corneal sensory fibers have mechanoreceptors that are sensitive to  
191 mechanical contact and generate one, or at most a few, nerve impulse(s) to convey acute  
192 sensations (Belmonte et al., 2004); these mechanoreceptors are all thinly myelinated A $\delta$  type  
193 nerves. In contrast to polymodal nociceptors, mechano-nociceptors are responsible for  
194 immediate and sharp sensations of pain induced by insults, including touching or scratching  
195 the corneal surface (Belmonte et al., 2017). Though TRPV1 is absent from A $\delta$   
196 mechanoreceptors (Murata and Masuko, 2006), another mechanosensitive ion channel  
197 (PIEZO2) may contribute to the sharp sensation of pain, which has been identified in corneal  
198 afferent neurons in the trigeminal ganglion (Alamri et al., 2015). Future studies are required  
199 to confirm its expression in the intraepithelial sensory nerves of the cornea.

200

201 The remaining 10% of corneal nerve fibers are A $\delta$  and C fibers, which have cold-sensitive  
202 nociceptors that respond to cool temperatures (less than about 33°C) at the ocular surface  
203 (Belmonte et al., 2017). It has been reported that corneal cold thermoreceptor activity is

204 enhanced with elevated tear osmolarity due to tear film evaporation, which may be associated  
205 with ocular discomfort in dry eye disease (Kovacs et al., 2016; Parra et al., 2010). In addition,  
206 inflammation can modulate the activity of corneal cold thermoreceptor by inhibiting impulses  
207 in response to cooling (Acosta et al., 2013). The transient receptor potential melastatin 8  
208 (TRPM8) cation channel plays an important role in corneal cold sensation (Parra et al., 2010).  
209 Through the response to cooling and elevated osmolarity, TRPM8-dependent impulses are  
210 responsible for regulating basal tear secretion (Parra et al., 2010).

211

212 Corneal nerves are not only involved in regulating tear production and blinking reflexes to  
213 maintain a lubricated and intact corneal epithelium, but also release numerous neurotropic  
214 substances, such as substance P (SP) and calcitonin gene-related peptide (CGRP), to  
215 modulate wound repair and assist in the maintenance of ocular surface health (Beuerman and  
216 Schimmelpfennig, 1980; Nishida, 2005). In mice, an age-related decline in corneal sensory  
217 nerve density has been reported (De Silva et al., 2019; Stepp et al., 2018), and reduced SP  
218 levels have also been described in the cornea and tear film of both aged mice and humans  
219 (Marco et al., 2018). SP released from corneal C fibers has an important role in maintaining  
220 corneal nerve structure and sensitivity (Marco et al., 2018), as well as promoting corneal  
221 wound healing in synergism with insulin-like growth factor (IGF) (Nagano et al., 2003;  
222 Nishida, 2005). A clinical trial demonstrated that eye drops containing SP and IGF-derived  
223 peptides induce a rapid re-epithelialisation of persistent epithelial defects in neurotrophic  
224 keratopathy (Yamada et al., 2008). The SP receptor is not only expressed on corneal sensory  
225 nerves but also on corneal immune cells, suggesting these neurotropic substances have dual  
226 neuro- and immune-mediated actions in the cornea. Although SP plays an important role in  
227 corneal wound healing by binding to its receptor in epithelial cells and attenuating the  
228 cytokine-chemokine network (Yanai et al., 2020), it may adversely affect ocular  
229 inflammation in some conditions (Suvas, 2017). In animal models of bacterial keratitis  
230 (*Pseudomonas aeruginosa*), SP causes an elevation of levels of pro-inflammatory cytokines  
231 (Foldenauer et al., 2012; McClellan et al., 2008), and application of a SP antagonist improves  
232 disease outcomes (Hazlett et al., 2007). Excessive release of SP may further promote  
233 leukocyte infiltration and delay the apoptosis of neutrophils, leading to neurogenic  
234 inflammation (Lasagni Vitar et al., 2022; Zhou et al., 2008). In addition, SP is also reported  
235 to promote inflammatory corneal neovascularisation, with increased infiltration of CD45<sup>+</sup>

236 leukocytes in mouse corneas after total de-epithelisation, alkali burn, or intrastromal sutures  
237 (Barbariga et al., 2018).

238

239 On the basis of an abundance of CGRP<sup>+</sup> nerves supplying the cornea, CGRP may also  
240 participate in corneal wound healing (He and Bazan, 2016). Regeneration of CGRP<sup>+</sup> nerves  
241 after corneal injury accelerated corneal epithelial healing (Cortina et al., 2012), and CGRP  
242 has been shown to enhance epithelial wound healing *in vitro* (Mikulec and Tanelian, 1996).  
243 Corneal sensory nerves release CGRP after electrical stimulation and heat or chemical  
244 irritants but not cold stimuli, indicating the release of CGRP may requires a vigorous  
245 stimulation of polymodal nerve endings (Belmonte et al., 2003). Similar to neuropeptide SP,  
246 the release of CGRP is believed to be pro-inflammatory leading to neurogenic inflammation  
247 (Belmonte et al., 2004; Lasagni Vitar et al., 2022). However, CGRP can also contribute to the  
248 resolution of corneal inflammation, as evidenced by the release of CGRP by macrophages  
249 and transformation of macrophages towards the anti-inflammatory phenotype in a mouse  
250 model of *P. aeruginosa* keratitis (Yuan et al., 2022).

251

252 The role of corneal sensory nerves in both maintaining epithelial maintenance and promoting  
253 wound healing is also closely linked with corneal epithelial stem cell function. In a mouse  
254 model of corneal nerve depletion induced by ophthalmic nerve electrocoagulation, limbal  
255 stem cell density was 75% lower than in control eyes, leading to impaired corneal epithelial  
256 healing (Ueno et al., 2012). The proliferative activity of corneal epithelial stem cells was 50%  
257 lower in the denervated eyes (Ueno et al., 2012). These results are consistent with the clinical  
258 observation of limbal stem cell deficiency in eyes with neurotrophic keratopathy, where  
259 patients are prone to developing persistent epithelial defects and non-healing ulcers (Delic et  
260 al., 2022; Dua et al., 2018). A recent study confirmed the dependence of corneal stem cells on  
261 corneal innervation, and demonstrated the critical role of sensory nerve TRPV4 in  
262 maintaining stemness of limbal basal cells (Okada et al., 2019). Impaired epithelial healing  
263 caused by sensory nerve damage can be rescued by TRPV4 gene introduction, which is  
264 accompanied by the recovery of stem cell function (Okada et al., 2019). Other neurotrophic  
265 factors, including nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF)  
266 and brain-derived neurotrophic factor (BDNF), may also play an important role in  
267 maintaining corneal epithelial stem cells (Qi et al., 2007).

268

269 In addition to its well described sensory nerve supply, the cornea is also considered to receive  
270 innervation from the autonomic nervous system; this topic has been covered recently in a  
271 review by Vitar et al (2022). The mammalian cornea receives sympathetic nerve fibers that  
272 predominantly originate from superior cervical ganglion (Marfurt and Ellis, 1993). This  
273 sympathetic innervation constitutes approximately 15% of the total corneal innervation in  
274 rabbits and cats (Morgan et al., 1987; Tervo and Palkama, 1978), but its contribution is  
275 considered relatively limited in humans (McDougal and Gamlin, 2015; Toivanen et al.,  
276 1987). Sympathetic nerve fibers were reported to be predominantly located in the limbal  
277 stroma (Ehinger, 1966; Marfurt and Ellis, 1993), but a recent study has shown that around  
278 10% of the central intraepithelial nerves in rats express sympathetic nerve markers (He et al.,  
279 2021). Parasympathetic fibers are also presented in the corneal stroma of rats and cats  
280 (Marfurt et al., 1998; Morgan et al., 1987), but these findings have not been confirmed in  
281 humans. Though the function of autonomic nerves in the cornea is not well understood, they  
282 may contribute to ocular surface health, corneal wound healing responses and/or neurogenic  
283 inflammation (Jones and Marfurt, 1996; Yun et al., 2016). The autonomic innervation in the  
284 cornea also releases neuropeptides including neuropeptide Y (NPY), acetylcholine and  
285 vasoactive intestinal polypeptide (VIP), which may participate in wound healing and  
286 angiogenesis (Ekstrand et al., 2003). VIP has also been shown to regulate inflammatory  
287 responses and nerve regeneration in the cornea (Zhang et al., 2020b). Animal studies have  
288 shown that sympathetic nerve activation inhibits corneal re-epithelialization and promotes  
289 neutrophil infiltration after injury, while parasympathetic nerve activation has the opposite  
290 effect (Xue et al., 2018). Sympathetic nerves may replace corneal sensory innervation in  
291 reactivated herpes stromal keratitis, which is dependent on CD4<sup>+</sup> T cell activation (Yun et al.,  
292 2022).

293

### 294 *3.2. Changes to corneal sensory innervation in ocular conditions*

295 Many ocular conditions have known associations with structural and/or functional  
296 abnormalities to corneal sensory nerves, which have been thoroughly reviewed relatively  
297 recently (Al-Aqaba et al., 2019). For the purpose of the present review, we focus on  
298 conditions that have been the subject of reports relating to neuroimmune interactions in the  
299 cornea.

300

301 *3.2.1. Dry eye disease*

302 Dry eye disease (DED) is one of the most common disorders affecting the ocular surface. The  
303 latest international Dry Eye WorkShop II (DEWS II) report from the Tear Film and Ocular  
304 surface Society acknowledged that corneal neurosensory abnormalities have an etiological  
305 role in DED (Craig et al., 2017). Using IVCN, some studies have reported reduced SBNP  
306 density in patients with DED (Cox et al., 2021; Giannaccare et al., 2019; Labbe et al., 2013),  
307 while others have reported that corneal nerve density remains unaffected (Hosal et al., 2005;  
308 Zhang et al., 2005). This discrepancy in the literature might, at least in part, be explained by  
309 the studies assessing patients with different stages, subtypes and/or severities of DED. In  
310 addition, variations in IVCN imaging methods, including the device used, number of images  
311 assessed, the method of image selection, and the location and depth of the corneal imaging,  
312 may contribute to reported differences in corneal nerve parameters in different studies (De  
313 Silva et al., 2017). For example, laser-scanning IVCN enables higher resolution images of  
314 intraepithelial nerves than the slit-scanning device, which may result in differential sensitivity  
315 to detecting corneal nerves *in vivo* using the two methods (Cruzat et al., 2017).

316

317 Tear hyperosmolarity and inflammation are considered key elements of the pathophysiology  
318 of DED (Bron et al., 2017). Tear hyperosmolarity can trigger innate immune and adaptive  
319 (CD4<sup>+</sup> T cell) responses in the cornea (Stern et al., 2013), which involves neuropeptide  
320 release from damaged nerves (Figure 5). In desiccating stress-induced models of DED in  
321 mice, inhibiting neurokinin-1 receptor (NK1R), the principle SP receptor, reduced DED  
322 severity by suppressing T helper cell mediated responses (Taketani et al., 2020; Yu et al.,  
323 2020). *In vitro*, substance P can also modulate T cell activation, selectively inducing Th17  
324 cell formation, or switching, from memory CD4<sup>+</sup> T cells to amplify inflammation (Cunin et  
325 al., 2011). Dendritic cells (DCs) play an important role in the activation of CD4<sup>+</sup> T cells  
326 (Schaumburg et al., 2011), and many studies report an increased density of DCs in patients  
327 with DED (Xu et al., 2021). In mice, hyperosmolarity can induce morphological and  
328 phenotypic maturation of corneal intraepithelial DCs within hours after topical exposure  
329 (Senthil et al., 2021). Together, these studies provide support for mechanistic links between  
330 nerve stimulation and recruitment and activation of corneal DCs, and also the  
331 immunomodulatory roles of neuropeptides in the cornea during DED.

332

333 Neuroinflammation in DED not only occurs in the cornea but can also involve the trigeminal  
334 ganglion and the trigeminal brainstem sensory complex. In a DED mouse model induced by  
335 unilateral excision of the extraorbital lacrimal gland and Harderian gland, reduced corneal  
336 intraepithelial nerves, decreased corneal mechanical hypersensitivity and increased  
337 spontaneous electrical activity of the ciliary nerve were observed (Fakih et al., 2019).  
338 Notably, increased pro-inflammatory markers were detected in the ipsilateral trigeminal  
339 ganglion and the trigeminal brainstem sensory complex (Fakih et al., 2019). Persistent ocular  
340 dryness and inflammation triggered corneal nociceptor activity that led to the development of  
341 corneal hypersensitivity and peripheral sensitization (Fakih et al., 2019). Similar results were  
342 observed in a topical benzalkonium chloride (BAK) model of ocular surface inflammation  
343 (Launay et al., 2016). The spread of inflammation from the cornea to the peripheral nervous  
344 system in experimental DED, and BAK-induced inflammation, lends further support to  
345 neuroimmune crosstalk in the cornea.

346

### 347 *3.2.2. Neurotrophic keratopathy*

348 Neurotrophic keratopathy (also known as neurotrophic keratitis) is defined as “a disease  
349 related to alterations in corneal nerves leading to impairment in sensory and trophic function  
350 with consequent breakdown of the corneal epithelium, affecting health and integrity of the  
351 tear film, epithelium and stroma” (Dua et al., 2018). Dua et al. recently reviewed  
352 neurotrophic keratopathy, and suggested a clinically relevant classification with three stages  
353 that relate to disease severity and prognosis: 1) Mild: epithelial changes only, without  
354 epithelial defect; 2) Moderate: epithelial defect without stromal defect; and 3) Severe: stromal  
355 involvement from corneal ulcer to lysis to perforation, with corneal hypo-  
356 aesthesia/anaesthesia (Dua et al., 2018). Many ocular and systemic diseases that involve  
357 corneal nerve impairment can induce neurotrophic keratopathy (Dua et al., 2018). One of the  
358 well-accepted theories is that impaired corneal nerves and reduced corneal sensation can  
359 result in diminished blink reflexes, reduced tear secretion and a deficit in neurotrophic  
360 support (Belmonte and Gallar, 2011; Wilson and Ambrosio, 2001). For example, corneal  
361 nerve injury is associated with reduced levels of the neurochemicals SP and acetylcholine;  
362 factors that have been shown to promote epithelial wound healing (Semeraro et al., 2014).  
363 Animal and clinical studies have demonstrated therapeutic effects with SP and IGF in

364 neurotrophic keratopathy (Nagano et al., 2003; Yamada et al., 2008). Therapeutic  
365 interventions using neurotrophins such as nerve growth factor (NGF) have shown great  
366 promise in treating neurotrophic keratopathy (Sacchetti and Lambiase, 2017), with recently  
367 conducted randomized, double-masked, vehicle-controlled trials confirming the efficacy of  
368 recombinant human NGF for treating neurotrophic keratopathy (Bonini et al., 2018;  
369 Pflugfelder et al., 2020).

370

371 Chronic inflammation triggered by corneal nerve disruption is also involved in the  
372 development of neurotrophic keratopathy. Disrupted tear secretion can induce tear  
373 hyperosmolarity and increased release of pro-inflammatory factors and neuropeptides,  
374 leading to neurogenic inflammation and progression of the disease (Baudouin et al., 2013;  
375 Dua et al., 2018). Activated collagenolytic enzymes in corneal inflammation can initiate  
376 stromal collagen melting, leading to the disease progressing to a moderate or severe stage  
377 (Fini et al., 1992). Studies have shown alterations to immune cell activity and cytokine  
378 release in diabetes-induced neurotrophic keratopathy (Lagali et al., 2018; Leppin et al.,  
379 2014).

380

### 381 3.2.3. Iatrogenic causes

382 Refractive surgery leads to corneal nerve degeneration as a result of transecting the sub-  
383 epithelial or anterior stromal nerves during the procedure. Several studies in animal models  
384 have explored the morphological and functional alterations to corneal nerves after  
385 experimental photorefractive keratectomy (PRK) (Bech et al., 2018; Medeiros et al., 2018).  
386 The regeneration of the SBNP starts shortly after PRK and reaches half the baseline density  
387 by day 15 in mice (Bech et al., 2018). In rabbits, nerve fibers sprout during the first month  
388 following PRK, however even after six months neither the SBNP nor the stromal nerves are  
389 fully recovered (Medeiros et al., 2018). In humans, 70%-80% of the SBNP remains absent at  
390 one month after PRK surgery and its regeneration takes much longer, with corneal nerve  
391 density only reaching half the pre-operative (baseline) levels after one year (Erie et al., 2005).

392

393 Laser-assisted *in situ* keratomileusis (LASIK) also leads to an 80% acute decrease in the  
394 density of nerves in the SBNP (Moilanen et al., 2008). Nerve regeneration can be observed in  
395 the central cornea at six months post-surgery, but it can take at least three years for the SBNP

396 to recover to its preoperative density, if at all (Calvillo et al., 2004; Erie et al., 2005).  
397 Importantly, it is well established that post-LASIK patients are prone to developing dry eye  
398 signs, including tear instability, decreased tear production and reduced corneal sensitivity (De  
399 Paiva et al., 2006); these clinical features have been linked to the damaged epithelial sensory  
400 nerves (Battat et al., 2001).

401

402 Laser epithelial keratomileusis (LASEK), which combines elements of LASIK and PRK, is  
403 milder in terms of its impact on SBNP density and corneal sensitivity compared to the  
404 LASIK at three and six months post-surgery; however, data at longer follow-up periods are  
405 not available (Lee et al., 2006). Recent advances in surgical procedures, such as small  
406 incision lenticule extraction (SMILE), where the refractive lenticule is extracted through a  
407 small incision without the creation of a corneal flap (Sekundo et al., 2011), aim to achieve  
408 more rapid post-operative corneal re-innervation (Denoyer et al., 2015). A recent study has  
409 reported that eyes subjected to SMILE procedures had significantly higher corneal nerve fiber  
410 density and nerve fiber length compared to those that had undergone LASIK at 4.1 years (Liu  
411 et al., 2020b); the individuals recruited in this study were of a relatively young age (mean of  
412 25 years at the time of surgery). The extent of corneal nerve regeneration after SMILE was  
413 age-dependent, with younger patients having more rapid nerve than older individuals (Li et  
414 al., 2021a).

415

416 Cataract surgery is another common iatrogenic cause of corneal nerve damage that occurs  
417 due to full thickness sensory nerve transection in the operated eye. Reduced corneal nerve  
418 fiber number and density, and increased nerve beading, have been described from analyses  
419 using corneal IVCN during the first three months after cataract surgery (De Cilla et al., 2014;  
420 Misra et al., 2015). A recent study demonstrated that corneal SBNP parameters, including  
421 nerve fiber density, fiber length and nerve total branch density, were decreased in both eyes  
422 in patients one month after unilateral cataract surgery, suggesting a contralateral eye effect on  
423 corneal nerve degeneration (Giannaccare et al., 2020). However, after the unilateral cataract  
424 surgery, the density of corneal DCs was described to not differ in either eye relative to  
425 preoperative levels (Giannaccare et al., 2020).

426



427 Penetrating keratoplasty (PK) is a surgical procedure that involves full thickness resection of  
428 the cornea, which includes severing the corneal nerve network. Corneal nerve fibers  
429 regenerate much slower after penetrating keratoplasty compared to cataract or refractive  
430 surgery, likely due to the widespread damage to axons during the procedure or the different  
431 corneal nerve conditions before the surgeries. At 12 months after PK, no SBNP fibers were  
432 detected by IVCN in a group of 20 patients (Darwish et al., 2007). In most patients,  
433 regeneration of the corneal SBNP and stromal nerves is observed to take one to 14 years after  
434 surgery, but no correlation was observed between the SBNP and stromal nerve trunk density  
435 (Al-Aqaba et al., 2012b). This finding suggests that the regenerated SBNP likely penetrated  
436 the donor graft directly from the host, and stromal nerves did not contribute to the epithelial  
437 innervation at this stage. Following PK, corneal sub-basal innervation can still be abnormal  
438 even after 40 years (Niederer et al., 2007), with reduced corneal sensation also existing for  
439 decades (Rao et al., 1985; Richter et al., 1996). In deep anterior lamellar keratoplasty  
440 (DALK), a partial graft procedure, regeneration of the SBNP and the recovery of corneal  
441 sensation are similar to PK (Lin et al., 2014; Zhang et al., 2013). Descemet's membrane  
442 endothelial keratoplasty (DMEK) and Descemet-stripping endothelial keratoplasty (DSEK),  
443 which are less invasive surgeries, cause acute reductions in SBNP density and corneal  
444 sensation, which are predominantly due to the corneal incision and descemetorhexis during  
445 surgery; however, most corneal nerve parameters return to baseline within four to 10 months  
446 post-operatively (Ahuja et al., 2012; Bucher et al., 2014).

447

448 Corneal collagen cross-linking (CXL) is a widely used treatment, intended to attenuate the  
449 clinical progression of keratoconus. The original protocol for the procedure involves removal  
450 of the corneal epithelium (giving it the name 'epithelium-off' CXL), thus leading to the  
451 destruction of the SBNP (Al-Aqaba et al., 2012a). Transepithelial CXL, where the corneal  
452 epithelium is retained *in situ* (so-called 'epithelium-on' CXL), results in significantly less  
453 damage to the SBNP (Al-Aqaba et al., 2012a; Caporossi et al., 2012). Physical removal of the  
454 epithelium is accepted as the main cause of corneal nerve damage from CXL procedures.  
455 Corneal SBNP density returns to pre-operative (baseline) levels at 12 months after  
456 epithelium-off CXL (Jordan et al., 2014), while complete regeneration of the SBNP after  
457 epithelium-on CXL was observed within six months (Bouheraoua et al., 2014). Corneal  
458 sensitivity also showed a faster recovery with the epithelium-on CXL method, compared to  
459 epithelium-off CXL (Spadea et al., 2015). Given the lower degree of corneal nerve damage

460 following the ‘epithelium on’ procedure, it is interesting to note that a recent systematic  
461 review reported that there was inadequate evidence that transepithelial CXL is more  
462 efficacious than epithelium-off CXL, in terms of disease progression, visual acuity and  
463 adverse events (Ng et al., 2021).

464

#### 465 3.2.4. Corneal infection

466 Ocular herpes simplex virus (HSV) infection is often characterized by mild manifestations  
467 such as blepharoconjunctivitis during primary infection, followed by long periods of latency  
468 in the trigeminal ganglion. Upon reactivation of the latent virus, recurrent infections can  
469 present as more severe corneal epithelial dendritic keratitis or a geographical corneal lesion  
470 (Lobo et al., 2019). Due to its neurotropic classification, HSV-1 pathophysiology is a topic of  
471 great interest in the ophthalmology, immunology and neuroimmunology fields. A decrease in  
472 corneal sensory nerve density and sensitivity has been described in a mouse model during  
473 primary corneal HSV infection (Chucair-Elliott et al., 2015). The virus can track in a  
474 retrograde direction, through the corneal nerve axons into the trigeminal ganglion, leading to  
475 long-term latency. Consistent with the concept of a connection between the two eyes by a  
476 retrograde neural and/or immune pathway (Lee et al., 2019), Hamrah *et al.* reported that in  
477 patients with unilateral HSV keratitis, both the infected and uninfected contralateral corneas  
478 showed similar alterations to corneal nerves, including decreased SBNP density and reduced  
479 corneal sensation (Hamrah et al., 2010). In addition, the nerve damage was evident in both  
480 the acute and chronic stages of HSV infection, suggesting that the infection can rapidly  
481 induce corneal neuropathy. A recent study revealed that the corneal SBNP in the HSV-  
482 affected eye regenerated slowly during the virus latency phase, however corneal nerve  
483 density did not reach the level of the healthy control group even after a follow-up period of  
484 29-46 months; in addition, the nerve regeneration did not result in an improvement in corneal  
485 sensation (Moein et al., 2018). These findings suggest that corneal HSV-1 infection leads to a  
486 severe loss of corneal nerves that takes years to regenerate, and that this neuropathy involves  
487 both eyes despite an often unilateral presentation.

488

489 Corneal nerve damage also occurs in patients with non-viral infectious keratitis including  
490 bacterial, fungal and *Acanthamoeba* keratitis (Cruzat et al., 2011; Muller et al., 2015). Fungal  
491 and *Acanthamoeba* keratitis cause a profound decrease in corneal nerve parameters compared

492 to epithelial herpetic keratitis (Kurbanyan et al., 2012), and the degree of corneal neuropathy  
493 is reportedly more severe than for bacterial keratitis (Cruzat et al., 2011). Following  
494 antimicrobial treatment, corneal nerve regeneration occurred after the resolution of infection,  
495 but did not reach baseline levels even at six months after cessation of treatment (Muller et al.,  
496 2015). Interestingly, a reduced density of corneal nerves and the presence of subclinical  
497 corneal immune cell infiltrates was observed in the contralateral (unaffected) eyes in patients  
498 with unilateral microbial keratitis. The observed contralateral eye effect, reminiscent of a  
499 sympathetic immune response, might be one explanation for the nerve damage in the  
500 unaffected eye (Cruzat et al., 2015). In a study of tear cytokines in patients with unilateral  
501 bacterial keratitis, interleukin (IL)-1 $\beta$ , IL-6 and IL-8 were elevated only in affected eyes  
502 while IL-10 and IL-17a were elevated only in unaffected contralateral eyes (Yamaguchi et  
503 al., 2014). The cytokine response in affected eyes might be pathogen-specific, whereas the  
504 unaffected eyes may be exhibiting a prophylactic host defense response by producing IL-  
505 17A.

506

507 The inflammation in the unaffected eyes might occur through a brainstem pathway (Launay  
508 et al., 2016). In a non-infectious benzalkonium chloride-induced unilateral corneal injury  
509 mouse model, the corneal nerve damage elicited neuron activation and inflammation in both  
510 the ipsilateral and contralateral sensory trigeminal complex in the brainstem, characterized by  
511 activated microglial cells and a release of pro-inflammatory mediators (Launay et al., 2016).  
512 A recent study also reported increased corneal nociceptor activity, in both the ipsilateral and  
513 contralateral eyes, in animals with mild unilateral corneal inflammation induced by UV  
514 exposure, as well as increased cold thermoreceptor activity bilaterally in a chronic tear  
515 deficiency model (Luna et al., 2021). The activation of projecting neurons and altered corneal  
516 sensory nerve activity may contribute to ocular pain and inflammation in the contralateral eye  
517 (Guzman et al., 2018). These contralateral eye studies provide strong evidence of the  
518 existence of functional neuroimmune interactions in the cornea that involve higher order  
519 structures, including the trigeminal ganglion and brainstem.

520

### 521 3.2.5. *Glaucoma*

522 Interest in corneal neuroimmune interactions in glaucoma patients stems from evidence that  
523 long term treatment of this disease with topical therapies is known to adversely affect the

524 ocular surface, mostly due to the presense of preservative agents in eye drop solutions. While  
525 most studies ascribe the ocular surface changes to the use of eye drops (Martone et al., 2009;  
526 Ranno et al., 2011), evidence of corneal nerve changes in patients with untreated glaucoma  
527 also exists (Jing et al., 2021). Structural alterations to corneal nerves, including lower nerve  
528 fiber length and branch number, have been reported in patients with untreated normal-tension  
529 glaucoma (Jing et al., 2021). However, patients with untreated primary open-angle glaucoma  
530 (POAG) share similar corneal SBNP features to healthy controls, suggesting that normal-  
531 tension glaucoma might be a primary neurodegenerative disease that is distinct from POAG  
532 (Jing et al., 2021).

533

534 The presence of corneal nerve damage in individuals with medically managed glaucoma  
535 provides evidence that topical, intraocular pressure-lowering medications can negatively  
536 effect corneal nerves (Villani et al., 2016). The commonly used preservative, benzalkonium  
537 chloride, induces the nerve damage due to its known neurotoxic effects (Sarkar et al., 2012).  
538 A recent study revealed that multi-therapy glaucoma patients had worse corneal nerve fiber  
539 parameters compared to those on unpreserved mono-therapy treatment regimens (Agnifili et  
540 al., 2022). Reduced corneal nerve fiber length was also observed in patients using preserved  
541 glaucoma mono-therapy compared to a healthy control group (Agnifili et al., 2022).  
542 Fogagnolo *et al.* (2015) investigated the effect of benzalkonium chloride exposure in a  
543 clinical trial and found that latanoprost (preserved prostaglandin analog), but not tafluprost  
544 (unpreserved prostaglandin analog), induced corneal SBNP changes, including altered nerve  
545 branching and nerve beading at 12-months of follow-up. Thus, it is becoming clear that  
546 preservative-containing topical treatments used to manage glaucoma can disrupt corneal  
547 nerve homeostasis. Furthermore, long-term use of such therapies increases the density of  
548 putative dendritic cells in the corneal epithelium (Marsovszky et al., 2014; Zhivov et al.,  
549 2010). However, whether the increase in corneal immune cells is associated with the  
550 neurotoxicity, or whether it is a direct response to the preservative agent(s), is unclear.

551

### 552 *3.3. Changes to corneal sensory innervation in systemic conditions*

553 As a densely innervated tissue, the cornea is vulnerable to damage from primary systemic  
554 diseases and chemotherapy-induced disorders that involve peripheral neurodegeneration  
555 (Campagnolo et al., 2013; Misra et al., 2017). Several systemic conditions have been

556 associated with subtle changes to the corneal epithelium and sensory nerves, which makes  
557 this tissue an attractive target for the identification of imaging biomarkers that may signal  
558 early stages of diseases. Chronic inflammatory autoimmune conditions have also been  
559 associated with diffuse corneal inflammation, and altered corneal nerve structure (Villani et  
560 al., 2008; Villani et al., 2010). IVCN allows non-invasive visualization of corneal nerves and  
561 immune cells, enabling early detection, as well as assessment of the progression, of  
562 peripheral neuropathy and response to treatments (Ferrari et al., 2013; Wang et al., 2015).

563

### 564 3.3.1. *Diabetes mellitus*

565 Diabetes mellitus is known to affect multiple ocular tissues, including the cornea; progressive  
566 damage to corneal epithelial cells and sensory nerves defines a condition known as diabetic  
567 keratopathy (Bikbova et al., 2018). A number of clinical studies have shown that both type 1  
568 and type 2 diabetes can negatively impact corneal innervation, with anatomical changes that  
569 include both decreased nerve fiber length and nerve fiber density (Jiang et al., 2016), and  
570 increased corneal nerve tortuosity (Ishibashi et al., 2012). Functional impairment has also  
571 been reported, including reduced corneal sensitivity to mechanical stimuli (Lv et al., 2014).  
572 Notably, decreases in corneal sensitivity are not congruent with the early morphological  
573 changes seen in the corneal sub-basal nerves (Messmer et al., 2010; Rosenberg et al., 2000),  
574 indicating IVCN may be able to detect corneal neuropathy at an early stage, before the onset  
575 of corneal sensory dysfunction.

576

577 The precise role of corneal immune cells in the early stages of diabetes-associated corneal  
578 neuropathy is unclear, with contrasting findings from mouse and human studies. Some animal  
579 and human studies suggest that diabetic corneal neuropathy (Leppin et al., 2014; Tavakoli et  
580 al., 2011) is associated with a higher density of presumed dendritic cells in the corneal  
581 epithelium. Lagali *et al.* (2018) reported that the proportion of so-called ‘mature’ dendritic  
582 cells was increased in individuals with impaired glucose tolerance and peaked within the first  
583 10 years of a type 2 diabetes diagnosis. Others report that experimental diabetes in mice  
584 reduces corneal dendritic cell density, and that this underpins delayed corneal epithelial  
585 wound healing responses after injury (Gao et al., 2016b). It is possible that the contributions  
586 of corneal immune cells to diabetic neuropathy relates to the type, stage and/or duration of  
587 disease. Alternatively, the recruitment and/or activation of corneal intraepithelial immune

588 cells may be a consequence of systemic inflammation due to chronic metabolic disturbance.  
589 In this respect, evidence exists from mouse high fat diet models, considered a model of pre-  
590 type 2 diabetes, whereby higher corneal immune cell density is observed in the early phases  
591 of the dietary intervention (Jiao et al., 2020b), with corneal immune cell changes apparent  
592 weeks prior to the onset of nerve pathology (Hargrave et al., 2020).

593

### 594 3.3.2. *Central nervous system diseases*

595 Corneal sensory nerve alterations have also been described in central nervous system (CNS)  
596 degenerative diseases. In a clinical study of individuals with moderate to severe Parkinson's  
597 disease, the density of the corneal SBNP was significantly lower in this population compared  
598 to healthy controls, despite no measurable difference in corneal sensitivity (Misra et al.,  
599 2017). Interestingly, a positive correlation was also observed between corneal SBNP density  
600 and Addenbrooke's Cognitive Examination-Revised scores, where higher scores are  
601 indicative of better cognitive function (Misra et al., 2017). Similar results of impaired corneal  
602 nerves and an association with cognitive function have been reported in individuals with mild  
603 cognitive impairment or dementia (Ponirakis et al., 2019).

604

605 Similar to these observations in humans, a number of animal studies have recently reported  
606 corneal nerve degeneration in mouse models of dementia. In transgenic mice overexpressing  
607 human non-mutated *tau*, corneal nerve degeneration occurred prior to cognitive deficits and  
608 peripheral neuropathy (Marquez et al., 2021). A recent study from our laboratory identified  
609 corneal nerve changes in a mouse model with CNS tauopathy, which was preceded by  
610 morphological alterations to the resident corneal epithelial dendritic cells at earlier stages of  
611 the disease (Jiao et al., 2020a). In a similar study involving P301L mice, which overexpress  
612 human *tau* protein, corneal nerve loss was greater in eight month-old mice compared to  
613 controls, and impaired corneal DC responses after corneal injury were observed in three  
614 month old P301L mice compared to controls (Li et al., 2021b). These alterations to corneal  
615 neuroimmune parameters in transgenic mice during the early stages of CNS degenerative  
616 disease are supported by human data, with altered corneal immune cell morphology described  
617 in patients with mild cognitive impairment, in the absence of significant corneal nerve  
618 abnormalities (Dehghani et al., 2020). Thus. Given the close relationship between corneal  
619 immune cells and sensory nerves, future studies evaluating corneal nerve changes as a

620 possible early biomarker of CNS degenerative disease should also consider including corneal  
621 immune cell analyses as an additional biomarker of interest.

622

623 Severe acute respiratory syndrome coronavirus 2 is a respiratory virus that causes coronavirus  
624 disease (COVID-19). Despite initially being considered a pulmonary disease, evidence of  
625 neurological manifestations affecting both the central and peripheral nervous systems have  
626 been reported (Severo Bem Junior et al., 2020; Wan et al., 2021a). Corneal IVCM imaging  
627 has revealed that four weeks after acute COVID-19 infection, patients with ongoing  
628 neurological symptoms have a lower corneal nerve fiber density and nerve fiber length, and a  
629 higher density of corneal DCs, compared to healthy controls (Bitirgen et al., 2021). A  
630 negative correlation between the severity of longCOVID and the extent of corneal nerve fiber  
631 loss has also been described (Bitirgen et al., 2021). Barros *et al.* (2022) reported similar  
632 findings among individuals who had overcome COVID-19; these patients showed lower  
633 corneal nerve fiber density and nerve fiber length compared to age-matched controls,  
634 accompanied by a higher density of immune cells in the central cornea. Overall, the  
635 percentage of patients with DCs in the central cornea was higher in COVID-19 patients  
636 (diagnosed > 6 months prior) with lower OSDI scores (<5 out of 100) (Barros et al., 2022). In  
637 what is a rapidly evolving clinical field, it is possible that IVCM imaging of corneal  
638 neuroimmune parameters will be useful as a surrogate marker of peripheral neuropathy, and  
639 also as a non-invasive means of assessing immune cell activation in peripheral tissues  
640 following COVID-19 disease, particularly in patients experiencing persistence of symptoms  
641 known as ‘long COVID’.

642

### 643 3.3.3. Autoimmune diseases

644 Various autoimmune diseases have been demonstrated to have a negative effect on corneal  
645 nerve features. In patients with rheumatoid arthritis, IVCM imaging showed more bead-like  
646 formations along corneal nerve axons, as well as more hyperreflective (presumably activated)  
647 keratocytes in the stroma (Villani et al., 2008). Likewise, decreased corneal nerve fiber  
648 density and increased tortuosity were observed in individuals with Grave’s orbitopathy, while  
649 those with active disease showed more hyperreflective keratocytes compared to those that  
650 had an inactive phase of disease (Villani et al., 2010). It is unclear if the corneal nerve  
651 impairments occur as a primary component of those autoimmune disorders, or if they are

652 secondary to ocular surface pathophysiology, including local immune responses (Shaheen et  
653 al., 2014).

654  
655 *3.3.4. Other systemic diseases*

656 A range of diseases that historically have been considered to not lead to corneal sequelae  
657 have been shown to manifest sub-clinical changes using IVCM imaging. For example, lower  
658 corneal nerve density and DED symptoms have been described in individuals with migraine  
659 (Kinard et al., 2015). People with migraine also experience more severe DED symptoms and  
660 ocular pain compared to those without migraine, suggesting DED symptoms in individuals  
661 with migraine may be driven by corneal nerve dysfunction (Farhangi et al., 2020). Damaged  
662 corneal nerve structure has also been described in individuals with fibromyalgia, including  
663 lower SBNP density and reduced stromal nerve thickness (Erkan Turan et al., 2018; Ramirez  
664 et al., 2015). In patients with human immunodeficiency virus (HIV) infection, corneal nerve  
665 fiber density was lower compared to controls (Kemp et al., 2017). Among these patients with  
666 HIV, lower corneal nerve density and a higher nerve tortuosity coefficient were identified in  
667 those with sensory neuropathy relative to those without (Kemp et al., 2017). However, these  
668 results need to be interpreted carefully as the patients with HIV-associated sensory  
669 neuropathy were older than those without sensory neuropathy, and age is an important factor  
670 in corneal nerve parameters (De Silva et al., 2019; Roszkowska et al., 2021).

671  
672 *4. Corneal immune cells*

673 Historically, the cornea has been regarded as an immune privileged tissue due, in part, to the  
674 absence of bone marrow-derived cells, except for intraepithelial DCs at the limbus (Gillette et  
675 al., 1982; Streilein et al., 1979). Subsequent studies have revealed that corneal immune  
676 privilege involves several mechanisms, including anatomical barriers, immunoregulatory  
677 processes and an immunosuppressive microenvironment (Hori et al., 2019). Further,  
678 advances in imaging techniques and the development of transgenic mice have contributed to  
679 an understanding that there are, in fact, distinct populations of resident immune cells  
680 distributed throughout the corneal epithelium and stroma (Figure 6).

681



682 4.1. Dendritic cells (DCs)

683 Some studies have historically classified corneal immune cells with long dendrites as  
684 'Langerhans cells' (Zhivov et al., 2005), which were generally assumed akin to epidermal DC  
685 populations that express Langerin (Valladeau et al., 2000). However, later data indicate that  
686 some dermal DCs also express Langerin, distinguished from the epidermal Langerhans cells  
687 by the expression of CD103 (Bursch et al., 2007). Similar to the skin, Langerin is expressed  
688 in human corneal DCs (Mayer et al., 2007), but it remains unclear whether they are related to  
689 the Langerhans cells or dermal Langerin<sup>+</sup> DCs. There is evidence that the murine corneal  
690 epithelium is endowed with a resident population of Langerhans cells, while there is also a  
691 minor population of Langerin<sup>+</sup> DCs in the stroma that are not Langerhans cells (Hattori et al.,  
692 2011). In this review, when commenting on mouse studies, we will refer to corneal  
693 intraepithelial, dendriform CD45<sup>+</sup> CD11c<sup>+</sup> cells as DCs.

694

695 Dendritic cells belong to a group of antigen-presenting cells (APCs) that typically express  
696 Class II major histocompatibility complex (MHC II) antigens. MHC II is required for the  
697 presentation of small, endocytosed peptides on the surface of APCs, in the context of co-  
698 stimulatory molecules CD80 and CD86. Presentation of antigenic peptides by APCs to naïve  
699 T-lymphocytes largely underpins the mechanism of adaptive immunity. The previously  
700 accepted dogma was that APCs were exclusively present in the peripheral cornea, but absent  
701 from the central cornea. Hamrah *et al.* (2002) earlier described that CD11c<sup>+</sup> DCs were  
702 present in both the central and peripheral corneal epithelium of BALB/c mice, with a  
703 centripetally decreasing density and that the DCs in the central and paracentral corneal  
704 epithelium of BALB/c mice were MHC II<sup>-</sup>, reflecting an immature status (Hamrah et al.,  
705 2003a; Hamrah et al., 2002).

706

707 In the presence of inflammation, resident corneal DCs can undergo maturation by  
708 upregulating MHC II (Hamrah et al., 2002) and co-stimulatory markers CD80 and CD86  
709 (Senthil et al., 2021). In more recent years, several other studies report that the majority of  
710 resident CD11c<sup>+</sup> intraepithelial DCs in both BALB/c and C57BL/6J mice co-express MHC II  
711 (Gao et al., 2016b; Jiao et al., 2019; Knickelbein et al., 2009; Leppin et al., 2014), thus it is  
712 becoming increasingly accepted that, at least in mice, resident corneal epithelial DCs are  
713 MHC-II positive in the steady state. Using transgenic Cx3cr1<sup>gfp/gfp</sup> knockin mice (Fig 3D&F),

714 which are deficient in Cx3cr1 receptor signaling, it was demonstrated that the presence of  
715 resident MHC II<sup>+</sup> DCs to the corneal epithelium was completely dependent on expression of  
716 the chemokine receptor Cx3cr1 (Chinnery et al., 2007). The dependence of corneal DCs on  
717 Cx3cr1 signaling was also validated using Cx3cr1 knockout mice (Chinnery et al., 2007).  
718 Cx3cr1 has also been shown to regulate the presence and sampling behaviour of resident  
719 intraepithelial immune cells to other mucosal sites, including the gut (Niess et al., 2005) and  
720 olfactory epithelium (Vukovic et al., 2010).

721

722 Similar to the reported presence of intraepithelial CD11c<sup>+</sup> MHC II<sup>+</sup> APCs in healthy mouse  
723 corneas (Knickelbein et al., 2014), an *ex vivo* analysis of human corneal tissue showed  
724 CD45<sup>+</sup> intraepithelial DCs that co-expressed CD11c<sup>+</sup> and HLA-DR<sup>+</sup>, with a centripetally  
725 decreasing density (Yamagami et al., 2005). Similar expression patterns of human corneal  
726 CD11c<sup>+</sup> DCs were also reported by Mayer *et al.* (2007). Thus, due to a close overlapping  
727 profile of DC phenotype and distribution to humans, murine models serve as effective  
728 systems to further investigate the phenotypes of corneal epithelial DCs and their roles in the  
729 process of corneal disease and wound healing, including nerve regeneration.

730

731 While macrophages are the predominant immune cell subset in the anterior corneal stroma  
732 (Brissette-Storkus et al., 2002; Chinnery et al., 2008; Liu et al., 2017), a minor population of  
733 DC subsets, including CD11c<sup>+</sup> CD11b<sup>+</sup> DCs, also reside in this corneal layer (Hamrah et al.,  
734 2003c). Morphologically, stromal CD11c<sup>+</sup> DCs are indistinguishable from macrophages,  
735 however they do appear to be preferentially associated with sites of nerve branch points,  
736 where the nerves traverse the basement membrane of the epithelium (Gao et al., 2016a).  
737 Similarly, macrophages in the anterior stroma have been observed to extend cellular  
738 processes into the basal epithelium, where they appear to interact with the sub-basal nerve  
739 plexus (Supplementary Figure 1 and Supplementary Video 1; Chinnery et al., 2017a). A  
740 sparse population of plasmacytoid DCs have also been described in the anterior corneal  
741 stroma, and are thought to produce interferon-gamma and regulate T cell immunity (Ochando  
742 et al., 2006; Reizis et al., 2011; Sosnova et al., 2005). Plasmacytoid DCs contribute to  
743 immune responses against corneal HSV-1 infection by promoting survival of regulatory T  
744 cells, indicating a critical antiviral role for these cells in the cornea (Jamali et al., 2020a).

745

746 4.2. *Macrophages*

747 Macrophages also reside in the healthy cornea. Although it is well-accepted that tissue  
748 macrophages originate from the bone marrow, resident macrophages can also derive from the  
749 yolk sac (Gomez Perdiguero et al., 2015). Macrophages participate in innate immune  
750 responses by phagocytosing debris and secreting a variety of inflammatory cytokines to assist  
751 with tissue remodeling and inflammation (Gordon and Martinez-Pomares, 2017). Following  
752 nerve injury, macrophages are recruited to the injury site, contributing to the removal of  
753 axonal and myelin debris, which is the source of inhibitory regeneration signals (Chen et al.,  
754 2015). In the mouse corneal stroma, CD45<sup>+</sup> macrophages are distributed throughout the  
755 anterior and posterior stroma (Figure 6), expressing typical markers including CD11b, Iba1  
756 and F4/80 (Chinnery et al., 2017a). Approximately 30% of corneal macrophages express  
757 MHC II molecules, which enable them to act as APCs in the cornea (Chinnery et al., 2008;  
758 Sosnova et al., 2005). In the human cornea, it has been shown that CD45<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>-</sup>  
759 resident macrophages are localized in the posterior corneal stroma (Mayer et al., 2007).

760

761 Macrophages are usually classified into pro-inflammatory phenotype (M1) and anti-  
762 inflammatory phenotype (M2) according to their distinct cytokine expression patterns and  
763 functions (Brown et al., 2012; Gordon and Taylor, 2005). In brief, M1 macrophages are  
764 activated by lipopolysaccharide, tumor necrosis factor- $\alpha$  and interferon- $\gamma$ , to participate in  
765 pro-inflammatory responses including anti-tumor immunity. M2 macrophages can be  
766 activated by specific cytokines, including IL-4, IL-10 and IL-13, to promote tissue repair and  
767 wound healing. Another marker, C-C chemokine receptor type 2 (CCR2), has been recently  
768 used to classify corneal macrophages in mice. Local (subconjunctival) depletion of either  
769 CCR2<sup>+</sup> or CCR2<sup>-</sup> macrophages delayed corneal wound healing after epithelial debridement,  
770 with corneal CCR2<sup>+</sup> macrophages sharing similar gene expression and function to M1  
771 macrophages and CCR2<sup>-</sup> corneal macrophages resembling M2 macrophages (Liu et al.,  
772 2017). Based on their phenotype and gene expression, CCR2<sup>-</sup> corneal macrophages likely  
773 originate from the yolk sac, similar to CCR2<sup>-</sup> macrophages and yolk sac-derived microglia  
774 (Liu et al., 2017). Using single-cell profiling and cell fate mapping of ocular immune cells,  
775 Wieghofer *et. al.*, demonstrated that despite most of the corneal macrophages exhibiting a  
776 molecular signature similar to bone marrow-derived monocytes, including continuous  
777 replenishment from the circulation during adulthood, there is also a contribution of  
778 embryonic precursors to resident corneal macrophages (Wieghofer et al., 2021). Thus, it is

779 becoming clear that stromal macrophages in the mouse cornea are heterogenous with respect  
780 to their phenotype, origin and contribution to wound healing.

781

#### 782 *4.3. Contribution of infiltrating immune cells to corneal nerve regeneration*

783 In addition to resident DCs and macrophages, infiltrating immune cells that are recruited  
784 following corneal injury have an important role in corneal inflammatory responses.  
785 Neutrophils are the dominant infiltrating cells in the early stage of inflammation,  
786 extravasating from the limbal vessels and migrating through the corneal stroma to the site of  
787 damage (Hanlon et al., 2014; Scapini et al., 2000). The migration of neutrophils relies on the  
788 local production of chemokines, such as the subset of CXC chemokines (Carlson et al., 2006;  
789 Lin et al., 2007). Animal studies have shown that neutrophils infiltrate the injured cornea in  
790 two phases; an initial phase within 12 hours and a second peak of neutrophil migration at 24  
791 to 48 hours that involves a small heat shock protein released by activated resident  
792 macrophages (Li et al., 2006; Oh et al., 2012). The recruited corneal neutrophils contribute to  
793 corneal re-epithelialization and angiogenesis (Gong and Koh, 2010; Li et al., 2006). Further,  
794 neutrophil accumulation is beneficial to corneal nerve regeneration after epithelial injury.  
795 Systemic depletion of neutrophils in wild-type mice resulted in a significant reduction in  
796 corneal nerve density, which was associated with reduced expression of vascular endothelial  
797 growth factor (VEGF) (Li et al., 2011a). This result was supported by evidence of impaired  
798 corneal nerve regeneration after injury in wild-type mice treated with anti-VEGF antibody (Li  
799 et al., 2011a). In addition to VEGF, other factors or cytokines may also participate in corneal  
800 nerve regeneration during the acute, neutrophil-intense phase of an inflammatory response. In  
801 neutropenic wild-type mice that have reduced neutrophil recruitment and delayed wound  
802 healing after corneal injury, topical application of recombinant IL-20 promoted corneal  
803 wound healing and nerve regeneration (Zhang et al., 2017). It is clear that neutrophils play  
804 key roles in corneal wound healing, and that an optimal balance of inflammatory cell  
805 infiltrates is required to achieve ideal rates of re-epithelialization and accompanying sensory  
806 nerve recovery.

807

808 In addition to resident corneal DCs and macrophages, a small population of  $\gamma\delta$  T cells are  
809 resident to the limbal epithelium of the steady state cornea; these cells comprise 90% of the T  
810 cell population at the limbus in mice (Li et al., 2007). Corneal  $\gamma\delta$  T cells have a dual effect on

811 ocular immunity. In one respect,  $\gamma\delta$  T cells play a vital role in maintaining ocular immune  
812 tolerance. Studies have shown that either TCR $\delta^{-/-}$  mice or mice treated with anti- $\gamma\delta$  TCR  
813 antibody fail to develop Anterior Chamber-Associated Immune Deviation (ACAID) (Skelsey  
814 et al., 2001; Xu and Kapp, 2001), suggesting that this subset of T cells contributes to the  
815 induction of ACAID to soluble antigen. Moreover, mice treated with anti- $\gamma\delta$  TCR antibody  
816 are at a higher risk of corneal allograft rejection due to the failure to develop ocular immune  
817 privilege (Skelsey et al., 2001). However, other studies have indicated that  $\gamma\delta$  T cells promote  
818 inflammation by inducing neutrophil infiltration (Li et al., 2007), which is critical for corneal  
819 wound healing. Another study has shown that IL-22 is a key factor mediating wound healing,  
820 and that topical treatment with IL-22 in TCR $\delta^{-/-}$  mice promotes epithelial wound closure (Li  
821 et al., 2011b). The  $\gamma\delta$  T cell-dependent inflammatory cascade involving VEGF and IL-17  
822 after corneal abrasion was found to be beneficial for promoting corneal nerve regeneration,  
823 providing evidence for corneal neuroimmune crosstalk following sterile injury (Li et al.,  
824 2011a).

825

## 826 *5. Corneal neuroimmune interactions*

827 Interactions between components of the nervous and immune systems have been investigated  
828 in many tissues, including the gut (Yoo and Mazmanian, 2017), lung and skin (Blake et al.,  
829 2019), and more recently in the cornea. The peripheral nervous system can respond rapidly to  
830 irritants and nociceptive stimuli, modulating local immune responses and, reciprocally,  
831 immune cells can affect neuronal function by releasing neurotransmitters and cytokines  
832 (Kawashima et al., 2015; Rosas-Ballina et al., 2011). Since the cornea is exposed directly to  
833 the external environment, its neural and immune systems are at the front line of responses to  
834 exogenous stimuli. Using advanced, post-acquisition image processing programs, high-  
835 resolution confocal microscopy images can be analyzed to quantify the degree of physical  
836 overlap between fluorophore channels (Figure 7). These approaches are based on the  
837 percentage overlap of user-generated rendered surfaces that localize to the structures of  
838 interest (Figure 7B-D). This quantitative approach is useful for measuring close  
839 neuroimmune interactions between corneal nerves and immune cells in 3D tissue  
840 preparations, however such findings should be interpreted with some caution, as unlike ultra-  
841 high resolution techniques such as serial block face scanning electron microscopy (Parlanti et  
842 al., 2020), confocal resolution limits do not allow the true visualization of cell contacts.  
843 Elucidating neuroimmune crosstalk in the cornea is important, and helpful, to better

844 understand the physiology and pathophysiology of corneal diseases, and potentially can be  
845 used to develop novel therapies aimed at promoting corneal nerve regeneration.

846

### 847 *5.1. Interactions between corneal nerves and intraepithelial immune cells*

848 Over the past decade there has been increasing evidence for reciprocal spatiotemporal and  
849 biochemical communication between corneal nerves and immune cells. In the healthy human  
850 cornea, most of the intraepithelial immune cells are localized to the level of the basal  
851 epithelial cells and SBNP (Zhivov et al., 2005). A recent study reported that the density of  
852 resident immune cells was positively correlated with corneal nerve branch density and total  
853 branch density in the normal human cornea (Colorado et al., 2019), suggesting the presence  
854 of corneal immune cells in healthy corneas may be beneficial to nerve health. Many diseases  
855 that involve corneal neuropathy have described concurrent alterations to intraepithelial  
856 immune cells and corneal sensory nerves. Corneal immune cell density has also been  
857 proposed as a potential marker of neuroinflammation in small fiber neuropathy (Kamel et al.,  
858 2019). Further to this, the degree of interaction between corneal epithelial immune cells and  
859 nerve axons, as determined visually using IVCM images, has provided evidence of at least  
860 spatial interaction in human corneas. By quantifying the number of corneal immune cells  
861 with at least one or more cellular processes in close contact with nerve fibres (Figure 7B), the  
862 interaction between these two constituents of the corneal epithelium can be measured and  
863 compared across populations with various diseases. Additional stratification of neuroimmune  
864 contacts can be performed by assessing the number of immune cells ‘without dendrites’ and  
865 cells ‘with dendrites’ that appear to cross or make contact with nerve fibres (Figure 7J)  
866 (Stettner et al., 2016). In a study of chronic inflammatory demyelinating  
867 polyradiculoneuropathy (CIDP), the number of DCs in close proximity to corneal nerve  
868 fibres was reported to be higher in patients with painful inflammatory neuropathy compared  
869 to both healthy controls and patients with painless neuropathy (Figure 7J) (Stettner et al.,  
870 2016).

871

#### 872 *5.1.1. Evidence for neuroimmune crosstalk in corneal homeostasis*

873 In steady-state conditions of the ocular surface, corneal nociceptors can detect changes in  
874 temperature, osmolarity and other elements of tear composition, to initiate the release of  
875 various neuromediators to maintain corneal homeostasis. Tear neuromediators including SP,

876 CGRP and VIP are present in healthy individuals (Yoon et al., 2022). SP has been reported to  
877 regulate reflex tear production and the expression of tight junction proteins in the corneal  
878 epithelium, suggesting a role in maintaining the barrier function of the ocular surface (Ko et  
879 al., 2009; Kovacs et al., 2005). Moreover, both human and mouse DCs express SP receptors,  
880 including NK1R and NK2R (Marriott and Bost, 2001), with evidence that NK1R signaling  
881 promotes the survival of DCs (Janelsins et al., 2009). Therefore, it is reasonable to speculate  
882 that SP contributes to maintaining resident DC populations in the densely innervated cornea,  
883 which regulates functional neuroimmune crosstalk.

884

885 Additional supportive evidence on the importance of neuroimmune crosstalk during ocular  
886 homeostasis stems from studies on ocular immune privilege. Ocular immune privilege is a  
887 phenomenon whereby the cornea and anterior chamber are able to limit local immune  
888 responses to preserve corneal integrity and function. One of the critical mechanisms is that  
889 the naïve T cell sensitization and effector T cell activation in the local lymphoid compartment  
890 is suppressed by regulatory T cells (Treg), leading to ocular surface immune quiescence  
891 (Amouzegar et al., 2016). In the aqueous humor, neuropeptides such as CGRP and VIP  
892 inhibit the activation of Th1 cells and macrophages to maintain a relative immunosuppressive  
893 microenvironment (Taylor, 2007). Corneal nerve integrity also plays an important role in  
894 ocular immune privilege. Corneal nerve injury can induce ocular surface CD11c<sup>+</sup> cells  
895 converting into contrasuppressor cells that inhibit Treg cells, leading to the loss of immune  
896 privilege (Neelam et al., 2018).

897

898 The expression of transient receptor potential (TRP) channels also serves as another form of  
899 functional connection between sensory nerves and immune cells. TRPV1, TRPV2 and  
900 TRPM8 are expressed on both corneal sensory nerves and tissue-resident immune cells  
901 express TRPV1 (Jiao et al., 2021; Khalil et al., 2016; Link et al., 2010). In the CNS, TRPV1  
902 is localized to the mitochondria of microglia, and promotes microglial migration (Miyake et  
903 al., 2015) and regulates glutamatergic signaling in neurons (Marrone et al., 2017). In the  
904 normal cornea, stromal macrophage expression of TRPV1 is evident, and is localized to  
905 CD68<sup>+</sup> lysosomes. After sterile injury, the proportion of TRPV1<sup>+</sup> macrophages is lower,  
906 suggesting a potential role for TRPV1 by resident macrophages in maintaining tissue  
907 homeostasis (Jiao et al., 2021). It is possible that corneal stromal macrophages, through

908 expression of TRPV1, modulate excitatory neurotransmission between corneal nerves and  
909 stromal cells, including keratocytes that express the glutamate receptor NMDAR1 (Sloniecka  
910 et al., 2015).

911

#### 912 *5.1.2. Evidence for neuroimmune crosstalk in diabetes mellitus*

913 Peripheral neuropathy is a common co-morbidity of patients living with diabetes. In  
914 individuals with type 2 diabetes, corneal nerve fiber density and branch density are lower,  
915 while the density of corneal DCs has been reported to be higher, relative to healthy control  
916 participants (D'Onofrio et al., 2021; Tavakoli et al., 2011). Lagali *et al.* (2018) reported a  
917 higher density of so-called 'mature' epithelial DCs (i.e., DCs with long dendritic processes)  
918 in individuals with type 2 diabetes, compared to healthy controls. Interestingly, the corneal  
919 DCs in patients with type 2 diabetes were more likely to be arranged in a cluster at the level  
920 of the SBNP, and were positively associated with tumor necrosis factor receptor super family  
921 member 9 protein levels in the plasma. In a pediatric population of individuals with type 1  
922 diabetes, early corneal nerve loss and a higher DC density were reported, compared to age-  
923 matched healthy controls (Ferdousi et al., 2019). While several clinical studies have reported  
924 concomitant changes to sensory nerves and immune cell populations in corneas of individuals  
925 with local or systemic pathologies, direct evidence for functional neuroimmune crosstalk, and  
926 its role in corneal homeostasis and pathology, mostly derives from animal studies involving  
927 transgenic and knock-out mouse models.

928

929 The synergistic interactions between corneal nerves and immune cells during epithelial  
930 wound healing in diabetic corneas has been reviewed recently (Yu et al., 2022). Animal  
931 models of type 1 and type 2 diabetes are commonly used to examine mechanisms of corneal  
932 neuropathy; several studies have demonstrated corneal nerve loss in mice and rats with  
933 established diabetes (Coppey et al., 2020; Leppin et al., 2014), and dietary-induced metabolic  
934 dysfunction that precedes type 2 diabetes (Alamri et al., 2019; Jiao et al., 2020b). In mice  
935 with type 1 diabetes, induced pharmacologically using streptozotocin (STZ), an increase in  
936 corneal DC density was associated with a lower corneal nerve fiber density (Leppin et al.,  
937 2014). In this study, close physical associations between mature corneal DCs and the SBNP  
938 were described using immunostained corneal flatmounts and confocal microscopy,  
939 suggesting a potential interaction between DC activation and corneal nerve fiber damage. In a



940 high-fat, diet-induced metabolic disturbance mouse model, our laboratory also observed a  
941 lower SBNP density and a higher density of intraepithelial DCs in the central cornea (Jiao et  
942 al., 2020b). In contrast, the sum length of the apically located SNT was similar in animals  
943 who consumed either a high-fat or standard diet (Jiao et al., 2020b). Given that most of the  
944 DCs are located in the basal corneal epithelium (Zhivov et al., 2005), these results provide  
945 evidence for neuroimmune interactions between epithelial DCs and sub-basal nerves, in the  
946 context of metabolic disturbance. Further analyses in this study identified that the percentage  
947 of mature DCs (MHC-II<sup>+</sup>) that were in close proximity to nerve axons in the SBNP was  
948 higher in the high-fat diet mice, relative to controls (Jiao et al., 2020b).

949

950 Although it is unclear whether resident corneal intraepithelial immune cells are  
951 neuroprotective or neurotoxic, or both depending on the condition, some clues have been  
952 provided in recent years. Gao *et al.* (2016b) reported that in STZ-induced diabetic mice with  
953 a corneal epithelial debridement wound, the number of resident and infiltrating DCs was  
954 lower and sensory nerve regeneration was delayed compared to animals without diabetes.  
955 This study also found that neutralization of ciliary neurotrophic factor (CNTF) delayed  
956 corneal sensory nerve regeneration after injury in the normal cornea. CNTF belongs to the  
957 neurotrophic factor family, and most of the CNTF in the cornea is produced by DCs (Gao et  
958 al., 2016b), suggesting that activated DCs may directly mediate nerve regeneration through  
959 production of neurotrophic factors, such as CNTF. When DCs were locally depleted,  
960 exogenous CNTF promoted reinnervation of the wounded cornea. In addition, blocking the  
961 CNTF-specific receptor induced corneal sensory nerve degeneration (Gao et al., 2016b).  
962 These findings provide evidence that DCs provide neurotrophic support to sensory nerves in  
963 the mouse corneal epithelium, and align closely with evidence of a neurotrophic role for  
964 intraepidermal Langerhans cells in cutaneous sensory nerve density and function (Doss and  
965 Smith, 2014).

966

### 967 *5.1.3. Evidence for neuroimmune crosstalk in acute corneal inflammation and sterile injury*

968 In addition to DCs and sensory nerves localizing to the corneal epithelium, DC processes  
969 appear to interact closely with sensory nerve axons and terminals (Gao et al., 2016a; Jamali et  
970 al., 2020b; Jiao et al., 2021; Jiao et al., 2020b; Leppin et al., 2014). Quantification of nerve-  
971 DC overlap has its challenges, as the complexity of both the cell processes and the abundance

972 of nerve axons can prove difficult when making binary decisions around cells that are, or are  
973 not, in contact with nerves. Using image analysis software to quantify the colocalization of  
974 rendered surfaces of fluorophore channels, we measured the degree of DC-nerve surface  
975 overlap in the normal mouse cornea, and in corneas following topical exposure to the TRPV1  
976 nerve antagonist, capsaicin, or the inflammatory cytokine TNF- $\alpha$  (Figure 7E-H). First, there  
977 was no regional difference in the degree of interaction between corneal DCs and nerves in the  
978 central versus the peripheral cornea. However, within 10 minutes of exposure to topically  
979 applied TNF- $\alpha$ , a known activator of corneal DCs, a higher degree of neuroimmune contact  
980 was observed, compared to topical application of capsaicin or vehicle controls. These data  
981 demonstrate the interaction between corneal DCs is dynamic, rapid and sensitive to changes  
982 in the inflammatory milieu of the ocular surface. Whether such physical neuroimmune  
983 interactions can be regulated by neuropeptide release, or if these dynamic interactions occur  
984 in human corneas, is so far unexplored.

985

986 Another study from our laboratory considered the relationship between corneal nerves and  
987 DCs in the context of local nerve trauma caused by sterile epithelial abrasion in mice. We  
988 observed that the degree of physical ‘contacts’, as measured by surface overlaps of rendered  
989 images of DCs and transient receptor potential vanilloid receptor 1 (TRPV1)-expressing  
990 corneal nerves, was higher in the injured epithelium compared to intact, uninjured controls  
991 (Figure 7I) (Jiao et al., 2021). Regarding the potential function of nerve-associated DCs,  
992 Parlanti *et al.* (2020) revealed that intraepithelial CD45<sup>+</sup> immune cells (presumed DCs)  
993 phagocytose and degrade axonal debris in a mouse model of corneal trephine wounds; this  
994 finding was evidenced by the presence of electron dense material, identified as lysosomes  
995 using scanning electron microscopy. Clearance of axonal debris after injury has been  
996 identified as an important processes in axonal regeneration in the peripheral nervous system  
997 (Dubovy, 2011), thus it is possible that corneal epithelial DCs participate similarly after  
998 corneal nerve injury.

999

1000 In addition to providing neurotrophic support to corneal nerves in systemic conditions that  
1001 are known to cause peripheral neuropathy, DCs also contribute to corneal nerve recovery  
1002 following local, sterile trauma. A recent study in our laboratory demonstrated that topical  
1003 application of decorin, a small leucine-rich proteoglycan, was associated with the recruitment  
1004 of corneal intraepithelial DCs at an early stage after sterile epithelial injury (i.e., six hours),

1005 and enhanced sensory nerve regeneration after one week of treatment (Wu et al., 2020). We  
1006 attribute this effect to decorin being able to modulate the expression of cytokines and/or  
1007 neurotrophins by the DCs. This DC-dependent neuroregenerative effect of decorin was  
1008 supported by findings in *Cx3cr1<sup>gfp/gfp</sup>* mice, which lack resident and infiltrating corneal  
1009 intraepithelial DCs (Wu et al., 2020). In these mice, decorin did not impart a therapeutic  
1010 benefit on corneal nerve regeneration following sterile injury (Figure 8).

1011

#### 1012 5.1.4. Evidence for neuroimmune crosstalk in dry eye disease

1013 Interactions between corneal sensory nerves and DCs are also evident in pathological  
1014 conditions that affect the ocular surface, including DED. Decreased corneal SBNP features,  
1015 including nerve fiber length, fiber density, fiber area and total branch density, have been  
1016 observed using IVCN in patients with DED, along with an increased density of presumed  
1017 DCs relative to healthy controls (Shetty et al., 2016). Notably, central corneal DC density in  
1018 patients with DED was positively correlated with ocular discomfort and SBNP features  
1019 including nerve fiber width, branch points and total fiber area, suggesting that increased  
1020 inflammatory cells and neural changes are associated with ocular discomfort experienced by  
1021 the patients with DED (Shetty et al., 2016). Tepelus *et al.* (2017a) also reported increased  
1022 corneal DC density and decreased nerve fiber density in patients with Sjögren's or non-  
1023 Sjögren's DED compared with age-matched control subjects. However, these authors  
1024 observed a negative correlation between corneal DC and nerve densities; it should be noted  
1025 that this correlation analysis included all study participants, including healthy controls. The  
1026 different findings in these two studies may be explained by the subtypes of DED studied  
1027 and/or the methods used for the IVCN image analyses. Patients with evaporative DED were  
1028 recruited in Shetty *et al.*'s study with corneal nerve features analyzed by automatic  
1029 ACCMetrics software. In Tepelus *et al.*'s study, over half of the participants were diagnosed  
1030 with dry eye due to Sjögren's syndrome, which is typically of the aqueous-deficient subtype,  
1031 and a semi-automated tracing program (NeuronJ) was used to quantify the corneal nerves.

1032

1033 In a mouse study, a decrease in the corneal SBNP density and an increase in DC density was  
1034 reported after exposure to environmental dry eye stress for three days (Simsek et al., 2018).  
1035 Using intravital multiphoton microscopy, Jamali *et al.* (2020b) investigated the functional  
1036 significance of physical connections between corneal nerves and DCs in a mouse model of

1037 DED. In this study, corneal DCs were found to be less frequently in contact with central and  
1038 peripheral corneal nerves during DED, compared with control corneas. Using time-lapse  
1039 intravital microscopy, this study also showed the DCs that were in contact with nerves  
1040 presented shorter track lengths, track speeds and displacement compared to those not in  
1041 neuronal contact, suggesting that corneal nerve interactions may directly regulate the  
1042 morphology and spatiotemporal dynamics of corneal DCs Jamali *et al.* (2020b). Moreover,  
1043 corneal DCs may indirectly protect sensory nerves in DED by inhibiting ocular inflammation  
1044 and regulating the expression of neurotrophic factors and neurotransmitters (Choi *et al.*,  
1045 2017). Increased inflammatory cell infiltration and decreased expression of calcitonin gene-  
1046 related peptide (CGRP), nerve growth factor (NGF) and SP were observed in DC-depleted  
1047 mice with experimental dry eye, and these changes were accompanied by a loss of  
1048 paracentral corneal nerves (Choi *et al.*, 2017). Thus, corneal DC-nerve interactions are  
1049 important for sensory nerve maintenance and corneal inflammatory regulation in DED.

1050

#### 1051 *5.1.5. Evidence for neuroimmune crosstalk in corneal infection*

1052 Pathological alterations to corneal immune cells and sensory nerves are well documented in  
1053 infectious keratitis, in particular HSV-1 and herpes zoster virus (HZV) infection. In patients  
1054 with unilateral herpes zoster ophthalmicus (HZO), a bilateral increase in corneal DC density  
1055 and decrease in SBNP length have been reported (Cavalcanti *et al.*, 2018). Corneal  
1056 denervation in the contralateral (uninfected) eye provides evidence for ocular inflammation-  
1057 associated nerve degeneration (Cavalcanti *et al.*, 2018). Although the mechanism underlying  
1058 sensory nerve degeneration with corneal HSV/HZV infection has not been fully elucidated,  
1059 the theory that viral replication is the only cause of nerve damage is debatable (Yun *et al.*,  
1060 2014). Due to its reliance on nerves, virus-induced neuronal death acts against the virus'  
1061 survival. Although inflammation plays a pivotal role in the tissue response to pathogens,  
1062 over-reactive and/or unresolved inflammatory responses can be harmful to local cells and  
1063 tissues. It has been proposed that inflammation from the viral infection may lead to corneal  
1064 nerve damage after HSV infection (Yun *et al.*, 2014). In a mouse model of acute HSV  
1065 infection, IL-6, produced during the early phase of the immune response, contributed to  
1066 corneal denervation, and neutralization of IL-6 partially preserved corneal nerve structure  
1067 (Chucair-Elliott *et al.*, 2016). In a rabbit model of corneal HSV infection, treatment with  
1068 topical pigment epithelial-derived factor (PEDF) plus docosahexaenoic acid (DHA) promoted  
1069 corneal nerve regeneration and improved resolution of the inflammatory response (He *et al.*,

1070 2017b). However, in a mouse model of HSV keratitis, despite the increased DC density and  
1071 corneal nerve damage after infection, local depletion of corneal DCs resulted in more severe  
1072 loss of corneal nerves (Hu et al., 2015). Gao *et al.* (2016a) reported similar results in sterile  
1073 injury model, where local depletion of corneal DCs resulted in a loss of nerve density and  
1074 delayed nerve regeneration. Likewise, our decorin-intervention study revealed that topical  
1075 decorin imparted a therapeutic benefit on corneal nerve regeneration in wild-type mice  
1076 following sterile injury but not in Cx3cr1<sup>gfp/gfp</sup> mice that lack resident and infiltrating corneal  
1077 intraepithelial DCs (Wu et al., 2020). Taken together, although alleviating inflammation  
1078 appears beneficial to corneal nerves, depletion of local corneal DCs may lead to a series of  
1079 changes in the local immune network, resulting in delayed nerve regeneration. Elucidating  
1080 the roles of DCs in corneal immune responses is necessary to more clearly define the  
1081 crosstalk between DCs and corneal nerves.

1082

1083 Divergent findings in the literature regarding the potential contribution of corneal DCs to  
1084 generating and resolving inflammatory responses may be explained by these immune cells  
1085 having a dual role in the pathophysiology of corneal infection. First, consistent with their  
1086 traditional function as APCs, corneal DCs initiate adaptive T-cell immune responses that in  
1087 turn can protect corneal nerves by inhibiting virus replication (He et al., 2017a; St Leger and  
1088 Hendricks, 2011). However, despite evidence that DCs have neuroprotective functions in the  
1089 cornea, over-reactive DCs and inflammation may delay corneal neuroregeneration (Wan et  
1090 al., 2022). Recent research from our laboratory has shown that CNS tauopathy is associated  
1091 with the activation of corneal DCs, which precedes the gradual loss of neighboring corneal  
1092 sensory nerves (Jiao et al., 2020). Another study also demonstrated that mice with unilateral  
1093 trephine-injured corneas had contralateral corneal nerve degeneration and DC infiltration  
1094 (Lee et al., 2019). Moreover, an increased number of mature DCs and effector lymphocytes  
1095 were observed in the draining lymph nodes, suggesting that immune cells might migrate  
1096 through the blood circulation to both corneas, causing further damage to the sensory nerves  
1097 (Lee et al., 2019).

1098

#### 1099 *5.1.6. Effects of corneal sensory nerve activation on immune cells*

1100 Given the intimate physical interactions between immune cells and sensory nerves in the  
1101 corneal epithelium, it is not surprising that immune cell homeostasis and inflammatory

1102 responses can be modulated by sensory nerve activation. Ablation of TRPV1-expressing  
1103 corneal sensory nerves increases corneal susceptibility to bacteria by modulating local innate  
1104 immunity, including reducing neutrophil bactericidal activity (Lin et al., 2021) and reducing  
1105 the capacity of corneal DCs to counter bacterial adhesion (Wan et al., 2021b). Sensory  
1106 neurons release mediators, including SP and CGRP, which directly attract and activate  
1107 immune cells (Ansel et al., 1993; Ding et al., 2008). Previous studies have reviewed the  
1108 important role of neuropeptides (e.g., SP, CGRP and VIP) in neuroinflammation at the ocular  
1109 surface (Bignami et al., 2016; Mantelli et al., 2010). There is evidence that following the  
1110 stimulation of polymodal nociceptors, the corneal sensory nerve endings release CGRP  
1111 (Belmonte et al., 2003), which can influence the development of local inflammation (Yin et  
1112 al., 2019). Increased levels of SP and VIP in the tears have also been described in association  
1113 with inflammatory responses in allergic conjunctivitis (Fujishima et al., 1997; Motterle et al.,  
1114 2006). Patients who experience contact lens discomfort also have elevated tear film levels of  
1115 the pro-inflammatory cytokine IL-17A, relative to those who have asymptomatic contact lens  
1116 wear (Downie et al., 2018; Gad et al., 2019). A recent study reported that unilateral eye  
1117 injuries in mice can lead to mucosal immune responses in the contralateral eye, which is  
1118 associated with TRPV1 activation in the injured eye and the release of SP in the opposite eye  
1119 (Guzman et al., 2018).

1120

1121 Neuropeptides can not only be released by sensory neurons, but also by immune cells  
1122 including DCs, macrophages, neutrophils and T cells (Suvas, 2017). These neuropeptides,  
1123 including SP and CGRP, can be detected in the tears of healthy humans, suggesting their role  
1124 in ocular surface physiology (Yoon et al., 2022). After corneal nerve damage, the release of  
1125 neuropeptides increases rapidly followed by the activation and infiltration of immune cells  
1126 (Barbariga et al., 2018). The role of neuropeptides in corneal neurogenic inflammation is  
1127 evidenced by immune cells largely expressing neuropeptide receptors (Morelli et al., 2020).  
1128 For example, in a mouse model of corneal alkali burn, there is a rapid increase of SP and its  
1129 receptor NK1R in the cornea, coinciding with an increased density of infiltrating leukocytes  
1130 (Bignami et al., 2014). In addition, SP can selectively induce T helper cell activation to  
1131 amplify inflammation, including immune cell infiltration and cytokine release (Cunin et al.,  
1132 2011). These inflammatory influxes result in a positive feedback loop that perpetuates the  
1133 inflammation and can lead to ocular surface diseases, such as DED and corneal  
1134 neovascularization. Sensory nerve-derived SP binds to NK1R on vascular endothelial cells

1135 and promotes vascular endothelial cell proliferation and tube formation (Liu et al., 2020a).  
1136 The involvement of SP in inflammatory corneal neovascularization has been identified in  
1137 both humans and animal models (Barbariga et al., 2018).

1138

1139 Based on the above consideration of the evidence, it is reasonable to hypothesize that the role  
1140 of DCs in modulating corneal reinnervation may depend on the stage of the inflammatory  
1141 response. At some stages, activated DCs may be important for promoting corneal nerve  
1142 regeneration by releasing neurotrophic factors. In conditions of chronic neuropathy, corneal  
1143 nerve damage may cause excessive DC activation, which could be regulated by either direct  
1144 neuroimmune contact, or indirectly by neurotransmitters.

1145

#### 1146 *5.2. Interactions between corneal nerves and macrophages*

1147 In addition to the role of corneal DCs in mediating neuroimmune interactions in the  
1148 peripheral nervous system (Feng et al., 2009), macrophages are also critically involved in  
1149 nerve regeneration. Macrophages have both pro-inflammatory and anti-inflammatory  
1150 phenotypes. There is evidence that macrophages contribute to Wallerian degeneration, a  
1151 process whereby distal portions of injured peripheral nerves progressively degenerate, with  
1152 the breakdown of axons and myelin (Chen et al., 2015; Namikawa et al., 2006). Following  
1153 peripheral nerve injury, macrophages are recruited by cytokines and chemokines that are  
1154 released by Schwann cells. Once at the site of nerve injury, macrophages participate in  
1155 clearing myelin and axonal debris, enabling axonal regeneration to occur (Chen et al., 2007;  
1156 Dubovy, 2011; Gaudet et al., 2011).

1157

1158 However, the mechanism in the corneal epithelium might differ, since most sensory nerves in  
1159 the corneal epithelium are unmyelinated and there are no Schwann cells surrounding  
1160 intraepithelial nerve axons. Instead, corneal epithelial cells are believed to function as  
1161 Schwann cells, including modulating macrophage recruitment by secreting cytokines and  
1162 regulatory factors, such as matrix metalloproteinase-9 (Pal-Ghosh et al., 2011; Stepp et al.,  
1163 2017). In the normal murine cornea, an intimate physical connection has been described  
1164 between corneal macrophages and peripheral stromal nerve trunks (Seyed-Razavi et al.,  
1165 2014). These peripherally located, nerve-associated macrophages were found to rapidly  
1166 respond to the damage of terminal axons in the central corneal epithelium, which was partly

1167 mediated by Cx3cr1 signaling. Our recent study revealed that stromal macrophages express  
1168 TRPV1 channels that are localized to CD68<sup>+</sup> lysosomes (Jiao et al., 2021). We observed a  
1169 lower proportion of TRPV1<sup>+</sup> macrophages in the injured cornea relative to homeostatic  
1170 conditions, suggesting that the expression of TRPV1 in resident macrophages is implicated in  
1171 corneal homeostasis (Jiao et al., 2021). Functionally, the consequences of TRPV1  
1172 expression by corneal stromal macrophages, and whether they contribute directly to nerve  
1173 homeostasis, is unclear. In other systems, TRPV1 expression by CD68<sup>+</sup> macrophages has  
1174 been reported in the synovium of healthy individuals and osteoarthritis patients (Lv et al.,  
1175 2021). In rats, activation of TRPV1 via intra-articular injections of TRPV1 agonists lowered  
1176 the proportion of M1 (inflammatory) macrophages in the synovium, and improved  
1177 osteoarthritis severity (Lv et al., 2021). These findings suggest that targeting TRPV1  
1178 expression may be a useful avenue for manipulating the profile of inflammatory macrophages  
1179 in tissues, which may be beneficial for promoting corneal nerve regeneration.

1180

1181 He et al. (2017c) reported that in diabetic mice with wounded corneas, topical treatment with  
1182 PEDF<sup>+</sup>DHA promoted sensory nerve regeneration and increased the recruitment of M2  
1183 macrophages. As previously discussed, M1 macrophages are broadly considered pro-  
1184 inflammatory, whereas M2 macrophages are immunosuppressive cells that contribute to the  
1185 repair of tissues and axons after injury (Chen and Bonaldo, 2013; Italiani and Boraschi,  
1186 2014). In a diabetic mouse model of corneal injury, topical treatment with netrin-1 (an axon  
1187 guidance factor) enhanced corneal nerve fiber regeneration, as well as M2 macrophage  
1188 transition (Zhang et al., 2018b). Similar results were obtained in another study from the same  
1189 laboratory, in which topical application of resolvin-D1 promoted corneal nerve regeneration  
1190 and enhanced M2 macrophage activation (Zhang et al., 2018c). The crosstalk between  
1191 corneal nerves and macrophages might be beneficial to resolving inflammation. *In vitro*  
1192 studies have shown that a synergistic effect between macrophages and sensory neuron-  
1193 induced neuropeptide CGRP release, which was also observed in mice with *P. aeruginosa*  
1194 keratitis (Yuan et al., 2022). The released CGRP regulated the transformation of M1  
1195 macrophages to the M2 subtype, promoting the resolution of corneal inflammation (Yuan et  
1196 al., 2022). Moreover, there is evidence that restoration of the distribution of corneal CCR2<sup>-</sup>  
1197 macrophages (similar to M2 macrophages) promotes corneal nerve regeneration through  
1198 increased secretion of neurotrophins, including brain-derived neurotrophic factor (BDNF),  
1199 NGF, neurotrophin (NTF)-3 and NTF-5 (Liu et al., 2018). The CCR2<sup>-</sup> macrophage subset



1200 preferentially express the  $\alpha$ -7 nicotinic acetylcholine receptor ( $\alpha$ 7nAChR). The activation of  
1201  $\alpha$ 7nAChR further enhances the expression of anti-inflammatory genes in the CCR2<sup>-</sup>  
1202 macrophage subset, which is necessary for corneal wound healing (Xue et al., 2018).

1203

1204 In our recent intervention study that investigated topical decorin for treating traumatic central  
1205 corneal nerve injury in mice, the density of corneal stromal macrophages was lower, and  
1206 nerve regeneration was higher, in decorin-treated corneas compared to controls (Wu et al.,  
1207 2020). This finding is not contradictory to previous studies, since the macrophages recruited  
1208 to the site of the injured nerves are expected to be a mix of phenotypes, and the total number  
1209 of macrophages is known to decrease with the resolution of inflammation (Lee et al., 2018).  
1210 For example, in the acute phase after partial sciatic nerve ligation, the M1 phenotype  
1211 dominates the macrophage population by day three post-injury. However, at day 14, the  
1212 percentage of M2 macrophages increases, with both subpopulations of macrophages equally  
1213 represented (Lee et al., 2018). A recent study using Macrophage Fas-Induced Apoptosis  
1214 (MAFIA) transgenic mice, in which macrophages can be depleted by intraperitoneal injection  
1215 of the dimerizer drug AP20187, corneal nerve structure and function were preserved after  
1216 corneal HSV infection (Chucair-Elliott et al., 2017). Therefore, inhibiting the pro-  
1217 inflammatory activity of M1 macrophages might be another means for promoting corneal  
1218 nerve regeneration during the early phase after injury.

1219

### 1220 *5.3. Interactions between corneal nerves and other infiltrating immune cells*

1221 Aside from DCs and macrophages, other immune cells such as neutrophils and T cells, that  
1222 are considered to typically not be resident to the cornea, are recruited to sites of nerve injury,  
1223 contributing to the inflammatory process (Liou et al., 2011; Moalem and Tracey, 2006). In a  
1224 corneal epithelial abrasion mouse model, the depletion of natural killer cells delayed corneal  
1225 nerve regeneration and increased the infiltration of neutrophils (Liu et al., 2012). Exaggerated  
1226 inflammatory responses may damage the corneal sensory nerves subsequent to cytokine  
1227 secretion. Corneal infiltration of inflammatory cells, and a higher expression of IL-17 and  
1228 IFN- $\gamma$  mRNA, occur in association with corneal nerve degeneration in mice with  
1229 experimental DED (Choi et al., 2017). Elevated tear IFN- $\gamma$  is also a feature of evaporative  
1230 DED in humans (Jackson et al., 2016).

1231

1232 Tian *et al.* (2018) reported that after topical application of PEDF in mice with corneal HSV  
1233 infection, the attenuation of neutrophil infiltration and reduced expression of IL-6, IL-1 $\beta$  and  
1234 TNF- $\alpha$  were all accompanied by reduced corneal nerve degeneration. These inflammatory  
1235 cytokines can modulate ion channels that concentrate in the nerve terminals (McMahon and  
1236 Koltzenburg, 1990), including reversing the operation of the sodium-calcium exchanger and  
1237 increasing calcium influx, which can otherwise promote axonal degeneration (Persson *et al.*,  
1238 2013). In addition, our laboratory's latest study demonstrated that the neuroregenerative  
1239 effect of topical decorin on the injured mouse cornea might be associated with fewer  
1240 infiltrating neutrophils in the early phase post-injury (i.e., within 24 hours). Moreover, the  
1241 decorin-induced neutrophil inhibition was absent in Cx3cr1<sup>gfp/gfp</sup> mice that lack corneal  
1242 epithelial DCs, indicating a potential interaction between DCs and neutrophils with topical  
1243 decorin treatment (unpublished data). Another recent study suggests that following excimer  
1244 laser annular keratectomy in thy1-YFP mice, YFP<sup>+</sup> bone marrow cells infiltrating the cornea  
1245 are myeloid-derived suppressor cells (MDSCs), expressing cell surface markers CD11b<sup>+</sup>Gr1<sup>+</sup>  
1246 (Sarkar *et al.*, 2013). An *in vitro* study also showed that MDSCs promoted the neurite growth  
1247 of a co-cultured trigeminal ganglion by secreting NGF (Sarkar *et al.*, 2013). Thus, the  
1248 attenuation of corneal neutrophil and NGF-secreting myeloid cells are possible targets for the  
1249 development of therapies aimed at improving nerve regeneration after injury (Sarkar *et al.*,  
1250 2013).

1251

1252 CD4<sup>+</sup> T cells also play a critical role in corneal nerve pathology. There is evidence that CD4<sup>+</sup>  
1253 T cells are required in HSV infection-induced neurogenic inflammation in the cornea. CD4-  
1254 depleted mice showed faster resolution of corneal inflammation and recovery of the blink  
1255 reflex at 20 to 70 day post-infection with HSV, which was not observed in wide type mice  
1256 (Yun *et al.*, 2014). The regeneration of the corneal nerve plexus and terminals was also  
1257 observed in CD4-depleted mice, indicating CD4<sup>+</sup> T cells may negatively affect corneal re-  
1258 innervation via neurogenic inflammation (Yun *et al.*, 2014). CD4<sup>+</sup> T cells also repress  
1259 sensory nerve growth in HSV-infected corneas via the production of VEGF (Yun *et al.*,  
1260 2020). These findings confirm that neurogenic inflammation can inhibit nerve regeneration,  
1261 or induce nerve degeneration, leading to a positive feedback and worsened corneal  
1262 neuropathy.

1263

1264 *5.4. Corneal neuroimmune interactions in ocular pain*

1265 Most corneal sensory nerves have polymodal nociceptors; their activation can lead to  
1266 sensations of discomfort and pain (Patapoutian et al., 2009). Pain can be classified into three  
1267 subtypes based on the anticipated underlying mechanisms: 1) nociceptive pain, stimulated by  
1268 noxious stimuli; 2) inflammatory pain, due to hypersensitivity and low thresholds caused by  
1269 inflammation; and 3) neurogenic pain, induced by nerve injury (Patapoutian et al., 2009).  
1270 Resident and infiltrating immune cells can be activated in both tissue injury-related  
1271 inflammation and neurogenic inflammation, and contribute to pain responses by releasing  
1272 pro-inflammatory cytokines. Local inflammation is a key component of many ocular  
1273 diseases, characterized by activated immune cells and increased release of pro-inflammatory  
1274 cytokines and neurotrophins. Many studies have demonstrated an upregulation in  
1275 inflammatory factors including IL-1, IL-3, IL-6, TNF- $\alpha$  and MMP-9 in DED (Bron et al.,  
1276 2017). These mediators can sensitize corneal nociceptors and lower their threshold for  
1277 activation, leading to the inflammation-related pain (Belmonte et al., 2017). Nociceptors may  
1278 then respond to normally innocuous stimuli (allodynia) in the absence of a noxious stimulus,  
1279 or show exaggerated responses to a noxious stimulus (hyperalgesia) (Patapoutian et al.,  
1280 2009).

1281

1282 Furthermore, inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  can directly stimulate  
1283 nociceptors and increase the membrane excitability of corneal nerves via activation of p38  
1284 mitogen-associated protein kinase (MAPK) (Binshtok et al., 2008; Wei et al., 2021). The  
1285 action potential firing further leads to an increased release of pro-inflammatory neuropeptides  
1286 including SP and CGRP, which is associated with a downregulation of TRPV1 receptor  
1287 expression (Yang et al., 2010). In addition, corneal sensory nerve damage can alter the  
1288 expression of transduction channels, including TRPV1, TRPA1 and TRPM8 (Staaf et al.,  
1289 2009), contributing to spontaneous neuropathic pain and a release of pro-inflammatory  
1290 neuropeptides (Baron, 2006). The pro-inflammatory neuropeptides and cytokines released by  
1291 activated nociceptors and immune cells result in a positive feedback loop, enhancing  
1292 spontaneous and stimulus-evoked nerve impulse activity in polymodal nociceptors.

1293

1294 *5.5. Role of aging in corneal neuroimmune interactions*

1295 Many studies have investigated the effects of aging on corneal innervation in rodents and  
1296 humans. Reduced SBNP density and corneal sensation were observed in aged mice (22  
1297 months) relative to younger animals (2 months) (De Silva et al., 2019; Stepp et al., 2018). In  
1298 rat corneas, the density of sensory nerves was similar between 10 and 56 week old animals,  
1299 but significantly lower by 120 weeks (He et al., 2021). Similarly in humans, increasing age  
1300 has been associated with a decline in corneal nerve fiber density and length, as well as lower  
1301 concentrations of the neuropeptides SP and CGRP in tears (Tummanapalli et al., 2020).

1302

1303 The immune system is generally affected by aging (Sadighi Akha, 2018). Age-related  
1304 inflammation, also known as inflammaging, plays a critical role in neurodegenerative  
1305 diseases, including Alzheimer's disease and Parkinson's disease (Scheiblich et al., 2020).  
1306 Aging can also affect immune cell phenotypes and their distribution at the ocular surface  
1307 (Golebiowski et al., 2020; McClellan et al., 2014). In mice, a higher proportion of CD11b<sup>+</sup>  
1308 CD11c<sup>+</sup> DCs was observed in aged mouse corneas compared to in younger eyes (Jung et al.,  
1309 2021). Among the DC subtypes, CD11b<sup>-</sup> CD11c<sup>+</sup> DCs induce cytotoxic CD8<sup>+</sup> T cell and Th1  
1310 responses, while CD11b<sup>+</sup> CD11c<sup>+</sup> DCs initial Th17 and Treg responses (Sun et al., 2020).  
1311 CD11b<sup>+</sup> CD11c<sup>+</sup> DCs contribute to the development of DED (Lee et al., 2012; Maruoka et  
1312 al., 2018), thus their increased presence in aging may contribute to the pathophysiology of  
1313 age-related DED and sensory nerve degeneration.

1314

1315 IVCN is also beginning to shed light on the effects of aging on human corneal immune cell  
1316 density and distribution. A higher ratio of central to mid-peripheral corneal intraepithelial DC  
1317 density was found in healthy humans of older age (Golebiowski et al., 2020). This age-related  
1318 change in corneal intraepithelial DC distribution might be associated with corneal nerve  
1319 alterations in the whorl region, which is also affected by aging (Badian et al., 2021). Healthy  
1320 volunteers aged over 50 years showed counterclockwise or non-rotatory corneal nerve whorl  
1321 patterns, accompanied by more dot-like features in IVCN images that are presumed to be  
1322 bulbous thickenings of the nerve fibers or possibly immune cells (Badian et al., 2021). The  
1323 relationship between inflammaging and corneal denervation in the cornea, and whether  
1324 corneal neuroimmune interactions are impacted by aging, requires further investigation.

1325

1326 *5.6. Autonomic nerves in corneal neuroimmune interactions*

1327 Despite only forming a minor contribution to corneal innervation, autonomic nerves also  
1328 participate in corneal neuroimmune interactions. After the loss of corneal sensory nerves,  
1329 sympathetic nerves invade into the stroma, forming large sprouts. The sympathetic nerve  
1330 fibers that hyperinnervate the cornea may promote HSV-associated inflammation by  
1331 releasing catecholamines (Yun et al., 2016). Depletion of CD4<sup>+</sup> T cells results in a lower  
1332 sympathetic innervation of the corneal stroma, indicating a central role for CD4<sup>+</sup> T cells in  
1333 the interactions between sympathetic nerves and inflammatory cells (Yun et al., 2022). In a  
1334 mouse model of corneal abrasion, activation of sympathetic nerves after injury inhibited  
1335 corneal re-epithelialization and promoted neutrophil infiltration and cytokine release, which  
1336 was associated with the polarization of corneal macrophages (Xue et al., 2018). The pro-  
1337 inflammatory effect of sympathetic nerves has also been reported in a corneal alkali burn  
1338 model, whereby the acute stimulation of corneal sensory nerves induced sympathetic  
1339 activation and noradrenaline release, which led to further corneal inflammation and  
1340 opacification (Lasagni Vitar et al., 2021).

1341

1342 The parasympathetic system also participates in corneal neuroimmune crosstalk, but likely as  
1343 a mechanism to balance the pro-inflammatory effects induced by sympathetic nerves. After  
1344 corneal injury, sympathetic nerve activation promotes inflammatory responses, while the  
1345 parasympathetic signaling inhibits neutrophil influx and the release of pro-inflammatory  
1346 cytokines to promote corneal epithelial regeneration (Xue et al., 2018). A possible  
1347 mechanism is that sympathetic and parasympathetic neurons respectively stimulate CCR2<sup>+</sup> or  
1348 CCR2<sup>-</sup> macrophages after corneal injury (Xue et al., 2018).

1349

1350 *5.7. Summary of corneal neuroimmune interactions*

1351 The current evidence suggests a reciprocal interaction between corneal immune cells and  
1352 sensory nerves (Figure 9). After corneal nerve injury, disrupted axons induce inflammatory  
1353 responses directly or by triggering the release of numerous neuromediators, leading to the  
1354 neurogenic inflammation. Both neurogenic inflammation and tissue inflammation can further  
1355 induce corneal nerve damage. Corneal inflammation is characterized by the infiltration of  
1356 neutrophils, transformation of macrophages into pro-inflammatory phenotypes (“M1” or  
1357 CCR2<sup>+</sup>), activation of CD4<sup>+</sup> T cells and the release of pro-inflammatory cytokines (e.g., IL-

1358  $1\beta$ ,  $IL-6$ ,  $TNF-\alpha$ ). However, there are also redress regulations existing in this picture of  
1359 reciprocal neuroimmune interactions. In some conditions, activated epithelial DCs and other  
1360 myeloid cells may have a neuroprotective effect on sensory nerves. These cells may directly  
1361 phagocytose and degrade axonal debris, or produce neuroprotective factors such as CNTF  
1362 and NGF. Moreover, anti-inflammatory macrophages (“M2” or  $CCR2^-$ ) also promote corneal  
1363 innervation by inhibiting further inflammation. Inhibiting the process of neuroinflammation  
1364 and/or augmenting neuroprotective mechanisms provides potential therapeutic strategies for  
1365 corneal neuropathy, but future studies are warranted.

1366

1367 It can be difficult to distinguish between neurogenic inflammation and tissue inflammation in  
1368 some corneal conditions, such as non-viral infection and diabetic keratopathy. Some  
1369 inflammatory components in neuroimmune interactions may have different roles in each  
1370 context (Figure 9). For example, during corneal wound healing, activated DCs can be  
1371 beneficial to nerve regeneration. A possible explanation is that in this scenario when the  
1372 corneal epithelium is lost and the tissue suffers a reduction in its DC population, recovery of  
1373 the epithelium and DC populations are foundational to the corneal nerve regeneration  
1374 process. In contrast, in inflammation-dominated conditions, such as diabetic keratopathy and  
1375 DED, DCs, as member of the innate immune system, likely produce pro-inflammatory  
1376 mediators that may contribute to corneal denervation. This concept of DCs having pro-  
1377 inflammatory and neurotoxic roles is consistent with a large body of evidence of elevated  
1378 corneal DC numbers, concomitant with fewer corneal nerves, in a range of corneal  
1379 inflammatory conditions (Cavalcanti et al., 2018; D'Onofrio et al., 2021; Shetty et al., 2016).  
1380 In the more complicated context of infection, DCs may present dual effects depending on the  
1381 course of the disease. DC-induced adaptive T-cell immune responses after infection are  
1382 beneficial for inhibiting virus replication and clearing bacteria (Frank et al., 2012), whereas  
1383 chronic inflammation can lead to further nerve degeneration. Similarly, nerve-derived SP can  
1384 promote epithelial wound healing after injury, providing an anatomical foundation for nerve  
1385 regeneration (Yanai et al., 2020). However, in inflammation-dominated corneal conditions,  
1386 excessive SP may induce T help cell activation to amplify inflammation (Lasagni Vitar et al.,  
1387 2022). Therefore, understanding the predominant neuroimmune interactions in different  
1388 corneal conditions and during steady-state is important for further translational studies.

1389

1390 Although our understanding of corneal neuroimmune interactions has increased in recent  
1391 years (Tables 1&2), many questions remain. In particular, there is a need for further  
1392 knowledge regarding how nerve-derived factors affect immune cell recruitment and function,  
1393 and how macrophage polarization in the cornea shapes nerve regeneration after injury.  
1394 Further investigations into the dynamic alterations of identified immune cell phenotypes in  
1395 the cornea, as well as mechanistic studies of specific therapies, are necessary to gain further  
1396 insights into the functional significance of corneal neuroimmune interactions.

Table 1. Key animal studies on corneal nerve and immune cell changes and their interactions (in the order of the publication year).

| First author (year)                                      | Animal model   | Intervention          | Changes to corneal nerves after intervention                  | Changes to immune cells after intervention   | Relationship between corneal nerves and immune cells |
|--|--|-----------------------|---|--|--|
| Namavari (2012)<br>(Namavari et al., 2012)               | Mouse model of corneal nerve-transecting lamellar flap surgery | Topical Semaphorin 7a | Increased corneal nerve length                                | Increased immune cell influx (CD45+)   | NR   |
| Liu (2012) (Liu et al., 2012)                            | Mouse model of corneal epithelial abrasion                     | Depletion of NK cell  | Delayed corneal nerve regeneration                            | Increased neutrophil influx  | NR   |
| Leppin (2014) (Leppin et al., 2014)                      | Mouse model of diabetes mellitus                               | NA                    | Decreased corneal nerve density                               | Increased corneal DC density   | Colocalization of nerve fibres and DCs               |
| Hu (2015) (Hu et al., 2015)                              | Mouse model of HSV keratitis                                   | Local depletion of DC | Decreased corneal nerve density                               | Depletion of corneal DCs   | NR   |
| Gao (2016) (Gao et al., 2016b)                           | Mouse model of diabetes mellitus                               | NA                    | Decreased corneal nerve density                               | Decrease corneal DC density  | NR   |
| Gao (2016) (Gao et al., 2016b)                           | Mouse model of corneal injury and local depletion of DCs       | Topical CNTF          | Increased corneal nerve density                               | Depletion of corneal DCs   | NR   |
| Gao (2016) (Gao et al., 2016a)                           | Mouse model of corneal epithelial debridement                  | Local depletion of DC | Decreased corneal nerve density                               | Depletion of corneal DCs   | DC dendrites cross nerve endings                     |
| Chucair-Elliott (2016)<br>(Chucair-Elliott et al., 2016) | Mouse model of HSV keratitis                                   | Topical dexamethasone | Retained corneal nerve density, increased corneal sensitivity | Decreased macrophage density<br>Decreased CD8+ T cell density                                    | NR   |
| Choi (2017) (Choi et al., 2017)                          | Mouse model of dry eye disease                                 | Local depletion of DC | Decreased corneal nerve density                               | Depletion of corneal DCs, increased infiltration of inflammatory cells (CD4+ CD19+ CD45+ CD11b+) | NR   |



| First author (year)                                   | Animal model   | Intervention                                  | Changes to corneal nerves after intervention | Changes to immune cells after intervention  | Relationship between corneal nerves and immune cells |
|---|--|---|--|---|--|
| He (2017) (He et al., 2017c)                          | Mouse model of diabetes mellitus and corneal injury            | Topical PEDF+DHA                              | Increased corneal nerve density              | Increased M2 macrophage density, decreased neutrophil density   | NR   |
| He (2017) (He et al., 2017b)                          | Rabbit model of HSV keratitis                                  | Topical PEDF+DHA                              | Increased corneal nerve density              | Increased neutrophil and macrophage density at 7 days, decreased neutrophil and macrophage density at 14 days | NR   |
| Chucair-Elliott (2017) (Chucair-Elliott et al., 2017) | MAFIA transgenic mouse model and HSV keratitis                 | AP20187 (to systemically deplete macrophages) | Increased corneal nerve density              | Depletion of macrophages  | NR   |
| Zhang (2018) (Zhang et al., 2018c)                    | Mouse model of diabetes mellitus and corneal epithelial injury | Topical resolvin D1                           | Increased corneal nerve density              | Increased M2 macrophage density   | NR   |
| Zhang (2018) (Zhang et al., 2018b)                    | Mouse model of diabetes mellitus and corneal epithelial injury | Netrin-1                                      | Increased corneal nerve density              | Decreased M1 macrophage density, increased M2 macrophage density, decreased neutrophil infiltration           | NR   |
| Simsek (2018) (Simsek et al., 2018)                   | Mouse model of dry eye   | NA  | Decreased corneal nerve density              | Increased DC density  | NR   |
| Tian (2018) (Tian et al., 2018)                       | Mouse model of HSV keratitis                                   | PEDF  | Increased corneal nerve density              | Decreased neutrophil density  | NR   |

| First author (year)                 | Animal model  | Intervention                         | Changes to corneal nerves after intervention   | Changes to immune cells after intervention                                  | Relationship between corneal nerves and immune cells              |
|-------------------------------------|---|--------------------------------------|--|---|---|
| Harris (2018) (Harris et al., 2018) | Mouse model of radiation keratopathy  | NA                                   | Decreased corneal nerve density  | Increased density of CD45+ immune cells, increased density of MHC II+ cells | Negative correlation between nerve density and CD45+ cell density |
| Liu (2018) (Liu et al., 2018)       | Mouse model of antibiotic-induced dysbiosis of gut microbiota and corneal epithelial abrasion | Fecal transplant/probiotic treatment | Increased corneal nerve density  | Increased density of CCR2-macrophages                                       | NR  |
| Liu (2018) (Liu et al., 2018)       | Mouse model of corneal epithelial abrasion  | Local depletion of CCR2- macrophages | Decreased corneal nerve density  | Depletion of CCR2-macrophages   | NR  |
| Lee (2019) (Lee et al., 2019)       | Mouse model of unilateral corneal injury  | NA                                   | Decreased corneal nerve density in both eyes   | Increased density of DCs and mature DCs (CD86+ or MHC II+) in both eyes     | NR  |
| Jiao (2020) (Jiao et al., 2020a)    | Mouse model of CNS tauopathy (transgenic rTg4510)   | NA                                   | Decreased density of corneal nerve axons, increased percentage of beaded nerve axons | Lower density of DCs<br>Higher proportion of CD86+ DCs                      | NR  |
| Wu (2020) (Wu et al., 2020)         | Mouse model of corneal epithelial abrasion  | Topical decorin                      | Increased corneal nerve length   | Increased DC density, decreased macrophage density                          | NR  |
| Jiao (2020) (Jiao et al., 2020b)    | Mouse model of high-fat diet (pre-diabetes)   | NA                                   | Decreased corneal nerve density  | Increased DC density  | Increased percentage of nerve-associated MHC-II+ cells            |

| First author (year)             | Animal model  | Intervention       | Changes to corneal nerves after intervention | Changes to immune cells after intervention                           | Relationship between corneal nerves and immune cells |
|---------------------------------|---|--------------------|--|--|--|
| Jiao (2021) (Jiao et al., 2021) | Mouse model of corneal epithelial abrasion                      | NA                 | Decreased TRPV1+ corneal nerve density       | Increase percentage of TRPV1+ nerve associated DC                    | Physical contacts between DC and nerve axons         |
| Wang 2021 (Wang et al., 2021)   | Mouse model of Type 1 diabetes with corneal epithelial abrasion | Topical calcitriol | Increased corneal nerve density              | Lower density of neutrophils, increased proportion of M2 macrophages | NR   |

Abbreviations: CCR2, C-C chemokine receptor type 2; CNTF, ciliary neurotrophic factor; CNS, central nervous system; DC, dendritic cell; DHA, docosahexaenoic acid; HSV, herpes simplex virus; MAFIA, Macrophage Fas-Induced Apoptosis; NA, not applicable; NK, natural killer; NR, not reported; PEDF, pigment epithelial-derived factor.

Table 2. Key human studies on corneal nerve and immune cell changes and their interactions (in the order of the publication year).

| <b>First author (year)</b>                 | <b>Disease/condition</b>                          | <b>Changes to corneal nerves compared to control group</b>                          | <b>Changes to corneal immune cells compared to control group, as described by the study authors</b> | <b>Relationship between corneal nerves and immune cells</b>             |
|--|---|---|---|---|
| Tavakoli (2011)<br>(Tavakoli et al., 2011) | Diabetes mellitus                                 | Decrease nerve fiber density, branch density and length; increased nerve tortuosity | Increased DC density  | NR  |
| Cruzat (2011)<br>(Cruzat et al., 2011)     | Infectious keratitis                              | Decreased nerve density, total number of nerves and branching                       | Increased DC density<br>Increased size and dendrites of DC  | Negative correlation between nerve density and DC density               |
| Villani (2013)<br>(Villani et al., 2013)   | Sjögren's syndrome dry eye and MGD                | Decreased corneal nerve fiber density, increased bead density and tortuosity        | Increased DC density  | NR  |
| Cruzat (2015)<br>(Cruzat et al., 2015)     | Unilateral infectious keratitis                   | Decreased nerve density, total number of nerves and number of branches in both eyes | Increased DC density in both eyes   | Negative correlation between nerve density and DC density               |
| Stettner (2016)<br>(Stettner et al., 2016) | Chronic inflammatory demyelinating polyneuropathy | Decreased nerve fiber density, nerve branch density and length                      | Increased density of DCs that contact nerve fibers, increased density of nondendritic cells         | NR  |
| Shetty (2016)<br>(Shetty et al., 2016)     | Dry eye disease                                   | Decreased nerve fiber density, fiber length and nerve branch density                | Increased DC density  | Positive correlation between DC density and corneal nerve fiber density |
| Choi (2017) (Choi et al., 2017)            | Dry eye disease                                   | Decreased nerve density, increased nerve tortuosity and beading                     | Increased DC density, process and area  | Positive correlation between DC process length and nerve density        |
| Tepelus (2017)<br>(Tepelus et al., 2017b)  | Mucous Membrane Pemphigoid                        | Decreased nerve density, increased nerve tortuosity                                 | Increased DC density  | NR  |
| Tepelus (2017)<br>(Tepelus et al., 2017a)  | Non-Sjögren's and Sjögren's syndrome dry eye      | Decreased nerve fiber density, increased nerve tortuosity                           | Increased DC density  | Negative correlation between nerve density and DC density               |

| <b>First author (year)</b>                | <b>Disease/condition</b>   | <b>Changes to corneal nerves compared to control group</b>                            | <b>Changes to corneal immune cells compared to control group, as described by the study authors</b> | <b>Relationship between corneal nerves and immune cells</b>                           |
|---|--|---|---|---|
| Bitirgen 2017 (Bitirgen et al., 2017)     | Multiple sclerosis   | Decreased nerve fiber density, fiber length and nerve branch density                  | Increased DC density  | NR  |
| Cavalcanti 2018 (Cavalcanti et al., 2018) | Unilateral HZO   | Decreased total nerve length, total nerve number and nerve branch number in both eyes | Increased DC density in both eyes, increased DC size and dendrite number in affected eyes           | Negative correlation between total nerve length and DC density                        |
| Bitirgen 2018 (Bitirgen et al., 2018a)    | Behcet's disease   | Decreased nerve fiber density and length  | Increased DC density  | NR  |
| Bitirgen 2018 (Bitirgen et al., 2018b)    | Fabry disease  | Decreased nerve fiber density and nerve fiber length, decreased corneal sensitivity   | increased DC density  | NR  |
| Aggarwal 2018 (Aggarwal et al., 2018)     | Fuchs' endothelial corneal dystrophy   | Decreased total nerve length and number   | Increased DC density  | NR  |
| Colorado 2019 (Colorado et al., 2019)     | Healthy human  | NA  | NA  | Positive correlation between corneal nerve branch density and resident DC density     |
| Dehghani 2020 (Dehghani et al., 2020)     | Mild cognitive impairment  | Decreased fiber length, fiber density and branch density, but not significant         | Similar DC density and higher DC field area and perimeter   | NR  |
| Klitsch 2020 (Klitsch et al., 2020)       | Fibromyalgia syndrome  | Decreased nerve fiber density and length  | No difference in DC density, decreased density of DCs that contact nerve fibers                     | NR  |
| D'Onofrio 2021 (D'Onofrio et al., 2021)   | type 1 diabetes mellitus, type 2 diabetes mellitus, and latent autoimmune diabetes of adults | Decreased nerve fiber density and length  | Increased DC density  | Negative correlation between nerve density and DC density in type 1 diabetes mellitus |

| <b>First author (year)</b>               | <b>Disease/condition</b> | <b>Changes to corneal nerves compared to control group</b> | <b>Changes to corneal immune cells compared to control group, as described by the study authors</b> | <b>Relationship between corneal nerves and immune cells</b> |
|--|--------------------------|--|---|---|
| Bitirgen 2021<br>(Bitirgen et al., 2021) | Long COVID               | Decreased nerve fiber density and length                   | Increased DC density  | NR  |

Abbreviations: CL, contact lens; DC, dendritic cell; HZO, Herpes Zoster Ophthalmicus; NA, not applicable; NR, not reported; MGD, meibomian gland dysfunction

## 6. Recent advances in promoting corneal nerve regeneration

Multiple ocular and systemic diseases can lead to corneal nerve degeneration. Severe cases of corneal nerve dysfunction can result in neurotrophic keratopathy, characterized by tear film abnormalities, corneal epithelial destruction and ulceration, and potentially corneal perforation. Despite the lack of epidemiological evidence regarding the prevalence of neurotrophic keratopathy, it is estimated that the incidence of neurotrophic keratopathy caused by herpetic keratitis and iatrogenic nerve damage is 1.22/10000 cases and 0.02/10000 cases respectively (Dua et al., 2018). Current clinical treatments for neurotrophic keratopathy primarily aim to promote corneal epithelial healing, but generally have limited capacity to encourage nerve regeneration or to restore corneal sensation (Pflugfelder et al., 2020). Although epithelial healing can provide anatomical structural support for corneal reinnervation and promote the secretion of neurotrophic mediators that enhance neurite survival (You et al., 2000), new treatments that are able to effectively promote corneal nerve regeneration and corneal sensitivity recovery are under investigation.

### 6.1. Neurotrophic/growth factors

Neurotrophic factors are a group of regulatory molecules that promote the survival of neurons and guide the growth of axons in the central and peripheral nervous systems. In skin, neurotrophic factors play important roles in cutaneous nerve regeneration as well as re-epithelialization and wound contraction (Ashrafi et al., 2016). Although several neurotrophic therapies have been shown to have positive experimental effects on corneal epithelial healing, very few demonstrate the ability to promote corneal reinnervation and restore corneal sensitivity in humans. Among them, NGF seems the most promising therapy for corneal reinnervation.

NGF, and its receptors Trk A (high affinity) and p75NTR (low affinity), are expressed in both healthy and injured corneas (Chung et al., 2013; Esquenazi et al., 2005; Qi et al., 2007; You et al., 2000). Lambiase *et al.* (1998) first reported outcomes from the application of topical NGF in patients with neurotrophic corneal ulcers. In this uncontrolled study, the authors described improved corneal healing in all 12 patient cases, and recovery of corneal sensitivity in most cases within 10 days to six weeks of treatment. Thereafter, a similar study with a larger sample population confirmed these results, however this study also lacked a

control group (Bonini et al., 2000). Topical recombinant human NGF (rhNGF) has been evaluated in two randomized, double-masked, vehicle-controlled trials in patients with neurotrophic keratitis (Bonini et al., 2018; Pflugfelder et al., 2020). The first study, a Phase 2 trial (NCT01756456), recruited 156 adult patients with moderate or severe neurotrophic keratitis, who were randomly treated with one of vehicle, 10 ug/ml, or 20 ug/ml rhNGF. Relative to vehicle, the rhNGF-treated groups showed a significantly higher proportion of patients who achieved corneal healing (defined as <0.5 mm lesion staining) after four or eight weeks of treatment. Another multi-center, randomized controlled trial (NCT02227147) confirmed the efficacy of rhNGF for treating neurotrophic keratitis. Both trials reported no significant safety concerns; most adverse events were ocular, mild, and judged to not be related to the study treatment. However, recent retrospective studies have reported more adverse events including difficulty sleeping and continued corneal thinning (Hatcher et al., 2021), as well as a rapid onset of corneal opacity identified as acute calcific band keratopathy (Qureshi et al., 2022). Neither of the two previous clinical trials showed a significant difference in corneal sensitivity (assessed using the Cochet-Bonnet esthesiometer) between rhNGF treatment and placebo, despite previous evidence that NGF can promote SBNP regeneration in a rabbit model after PRK and improve corneal sensitivity recovery in patients after LASIK (Esquenazi et al., 2005; Joo et al., 2004).

Given that immune cells can secrete growth factors such as NGF that have potential effects on corneal nerves, they may be an important component of the corneal neuroimmune interactions that underlie immune-mediated nerve repair. There is thus scope to target the function of immune system cells to repair injured nerves in the cornea. For example, in mice with excimer laser annular keratectomy, immune cells derived from bone marrow infiltrate the cornea (Sarkar et al., 2013); *in vitro* experiments demonstrate that these cells promote neurite growth by secreting NGF (Sarkar et al., 2013). Another recent study revealed that VEGF produced by CD4<sup>+</sup> cells suppressed corneal nerve regeneration in HSV-1 infected mice, and depletion of the CD4<sup>+</sup> cells promoted reinnervation of corneal sensory nerves (Yun et al., 2020). These findings provide evidence for targeting cells of the immune as a potential therapeutic approach for corneal nerve regeneration. A similar therapeutic strategy, involving the targeting of immune cells has been described in a preclinical model of optic nerve injury, using the transplantation of activated macrophages to promote optic nerve regeneration (Lazarov-Spiegler et al., 1996).



There is mounting evidence that a variety of other neurotrophic factors, and their receptors, exist in the cornea, mainly in epithelial cells and stromal keratocytes (Chung et al., 2013; You et al., 2000). The topical application of neurotrophic factors, including glial cell-derived neurotrophic factor (GDNF), PEDF and CNTF, can promote corneal nerve regeneration in diabetic mice (Di et al., 2017a; Gao et al., 2016b; Tian et al., 2018). A novel neurotrophic factor, mesencephalic astrocyte-derived neurotrophic factor (MANF), is highly expressed in the healthy mouse cornea, but not in diabetic mouse corneas during homeostasis and corneal injury. Application of recombinant human MANF was reported to accelerate corneal reinnervation and improve corneal sensitivity in normal and diabetic mouse corneas after injury (Wang et al., 2020). Other growth factors are under investigation, including VEGF, which has been shown to improve corneal nerve regeneration in normal and diabetic mice after corneal injury (Brash et al., 2019; Di et al., 2017b). Subcutaneous injection of insulin-like growth factor-1 (IGF-1) in diabetic rats has also been found to preserve corneal nerve innervation (Aghanoori et al., 2019).

Semaphorins are a class of membrane and secreted proteins that are identified as repulsive guidance molecules for axonal growth cones (Luo et al., 1993; Pasterkamp, 2012). In a murine dry eye model, topical application of selective Semaphorin 3A (Sema3A) inhibitor preserved corneal SBNP density and corneal nerve sensitivity, as well as the expression of TRPV1 (Yamazaki et al., 2017). The neuroregenerative effect of Sema3A inhibition was also demonstrated in a mouse corneal transplantation model (Omoto et al., 2012). However, in contrast, a study by Zhang *et al.* (2018a) reported that Sema3A promoted corneal nerve regeneration after epithelial debridement. These conflicting results might be explained by differences in the administration of the various agents. Specifically, Zhang *et al.* used a slow release delivery method, achieved by inserting pellets containing Sema3A into an intrastromal micropocket. The Sema3A inhibitor in the other two studies (Omoto et al., 2012; Yamazaki et al., 2017) was applied topically or by subconjunctival injection. Further studies are needed to elucidate whether or not Sema3A is neuroprotective in the context of corneal injury.

## 6.2. Anti-fibrotics

The corneal stromal microenvironment is considered to influence corneal nerve regeneration, due to the expression of neurotrophins and cytokines, as well as the development of fibrosis after injury (Chaudhary et al., 2012; Hamrah et al., 2003b). The scarring response has an inhibitory effect on nerve axon growth, which is associated with accumulation of scar-derived axon growth inhibitory ligands (Davies et al., 1999; Kawano et al., 2012). A recent study reported that activation of myofibroblasts in the corneal stroma inhibited nerve regeneration in a cat model of photorefractive keratectomy (PRK) (Jeon et al., 2018). The myofibroblasts showed diverse effects on axon growth, secreting transforming growth factor beta 1 (TGF- $\beta$ 1), which inhibits neurite outgrowth via collapsin response mediating protein 2 (CRMP2) signalling (Jeon et al., 2018). Topical application of the anti-fibrosis agent mitomycin C (MMC) after PRK has been shown to accelerate sensory nerve regeneration and decrease myofibroblast differentiation (Hindman et al., 2019; Jeon et al., 2018). However, Medeiros *et al.* (2018) reported corneal nerve toxicity from MMC application after PRK, consistent with the neurotoxic effects of this agent (Sui et al., 2014). MMC acts non-selectively against cell proliferation and differentiation, leading to a potential risk of various corneal complications, including delayed epithelial healing and endothelial decompensation (Arranz-Marquez et al., 2019). Anti-fibrotics with more specific targets and less adverse effects might be considered as potential therapeutic strategies for corneal nerve regeneration, however further research is required to expand our understanding of the interactions between corneal fibrosis and nerve regeneration.

## 6.3. Autologous serum and platelet-rich plasma eye drops

Autologous serum and platelet-rich plasma eye drops provide a cocktail of neurotrophins, neuropeptides and growth factors that may be beneficial for supporting corneal re-innervation. Several studies have shown that autologous serum tears (20%), dosed eight times daily for one to eight months, can improve corneal SBNP regeneration in patients with corneal neuropathy (Aggarwal et al., 2019; Aggarwal et al., 2015). Sjögren syndrome-related DED patients treated with autologous serum tears (five times daily for one year) showed reduced number of sub-basal nerve branches and a reduced degree of beading compared to those treated with artificial tears (Semeraro et al., 2016). However, no changes to corneal intraepithelial DC density were observed after treatment with autologous serum tears

(Aggarwal et al., 2015). In addition, application of human platelet lysate for 14 consecutive days enhanced corneal nerve regeneration in wounded rat corneas, and human platelet lysate promoted neuronal growth in an *in vitro* study (Huang et al., 2021). A randomized controlled trial evaluating the efficacy and safety of cord blood eye drops in patients with neurotrophic keratitis is ongoing (NCT03084861).

#### *6.4. Omega-3 fatty acid supplementation*

Long-chain omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and DHA, have anti-inflammatory and neuroprotective properties (Calder, 2017; Downie et al., 2018; Robson et al., 2010). There is pre-clinical evidence that topical application of resolvin-D1, a metabolite of DHA, can promote corneal nerve regeneration and restore corneal sensitivity in diabetic mice with corneal epithelial abrasion (Zhang et al., 2018c). Several studies have shown the DHA enhances the neuroprotective efficacy of other neurotrophic factors, including NGF and PEDF, for murine corneal reinnervation (Esquenazi et al., 2005; He et al., 2017b).

Menhaden oil, a natural source of omega-3 polyunsaturated fatty acids, can preserve corneal nerve fibers in chronic obese and type 2 diabetic rats (Coppey et al., 2020). A randomized controlled trial from our laboratory reported improvements in corneal SBNP parameters in DED patients, relative to placebo, after a moderate daily dose of oral omega-3 fatty acid supplementation for 90 days (Chinnery et al., 2017b; Deinema et al., 2017). Another single-arm, open label trial reported that 12-months of supplementation with a moderate-dose of oral omega-3 fatty acids led to increased corneal nerve fiber length in patients with type 1 diabetes (Lewis et al., 2017). Overall, participants tolerated the omega-3 fatty acid supplementation well, with no significant adverse events. Considering the totality of the available evidence, a recent systematic review and meta-analysis by our group concluded that there was low certainty evidence for a neuroprotective effect on peripheral nerves with systemic omega-3 fatty acid supplementation, and that further research was required to clarify the role of this intervention as a potential therapeutic for peripheral nerve disease (Zhang et al., 2020a). Participants' baseline omega-3 fatty acid dietary intake is an important potential modifier of the therapeutic efficacy of omega-3 fatty acid interventions, but has not been routinely measured in clinical trials of this intervention (Downie et al., 2019; Zhang and

Downie, 2019). Addressing these evidence gaps and lending further support to a role for oral omega-3 supplementation in treating corneal neuropathy, a recent randomized, double-masked, placebo-controlled trial completed by our team evaluated the effects of six months of oral omega-3 fatty acid supplementation on corneal nerve parameters, with adjustment for the baseline systemic Omega-3 index; this study found a significant corneal neuroregenerative effect with the omega-3 intervention in patients with type 1 diabetes (Britten-Jones et al., 2021). The observed neuroregenerative effect of oral omega-3 fatty acid supplementation might be, at least in part, associated with an anti-inflammatory effect. Consistently, treatment with topical DHA, neuroprotectin D1 and resolvins in preclinical studies have also demonstrated improvements in corneal nerve density in concert with an attenuation of local inflammation (Cortina et al., 2013; He et al., 2017b; Zhang et al., 2018c).

### *7. Future directions and perspectives*

Neuroimmune crosstalk in the cornea has attracted much attention in recent years. Despite considerable progress towards understanding the intricate interplay between corneal immune cells and sensory nerves, a range of complex questions and challenges remain. For example, how does the presence and activation of corneal DCs and the physical interactions between immune cells and sensory nerves affect corneal nerve health? Although the majority of clinical studies report a negative association between the number of corneal epithelial immune cells and nerve density, usually in the context of disease, animal studies using corneal DC depletion techniques clearly show that the absence of intraepithelial DCs is detrimental to corneal nerve health. It is important to note too, that the vast majority of clinical studies using IVCN images to measure corneal immune cells tend to broadly classify all intraepithelial immune cells located alongside the sub-basal nerve plexus as ‘dendritic cells’. In some cases, there is progress towards the classification of corneal intraepithelial immune cells based on morphology, and even a shift in the terminology from Langerhans cells or dendritic cells to ‘immune cells’, which is appropriate for now. It is possible that, with time, the immunological identity of these morphological subtypes will be further refined, in which case a re-assessment of studies examining corneal neuroimmunology may be required to improve our understanding of the relationship between corneal nerves and specific cellular populations. Future research, taking advantage of the local depletion or functional inhibition of specific immune cell subsets, available with the use of animal models, is required to grow this field of research.

Another question that remains is whether the number of epithelial immune cells, and/or the ratio of morphological subtypes (namely ‘mature’ immune cells with dendrites, and so-called ‘immature’ cells without dendrites) in an individual’s cornea is associated with later nerve damage and/or recovery. This information could provide useful predictions about the expected degree of nerve damage, and the relative rate of neuroregeneration, in patients undergoing surgical procedures, such as cross-linking and refractive surgery, that are well known to cause long-standing corneal nerve damage. Perhaps more boldly, the neuroregenerative capacity of corneal epithelial DCs, at least in animal models for now, may represent a novel therapeutic strategy for cell-based delivery of biological factors, including CNTF, that may promote local nerve regeneration *in vivo*.

Another underexplored area of corneal neuroimmunology relates to the macrophage subpopulations in the corneal stroma, and whether they contribute differentially to intraepithelial sensory nerve degeneration and repair. Although not directly located in the epithelium, nerve-associated macrophages are positioned along the nerve trunks in the stroma, and appear to rapidly dissociate within hours of epithelial nerve injury (Seyed-Razavi et al., 2014). Later, macrophages likely contribute to overall resolution of inflammation after injury, which may indirectly influence nerve recovery. Thus it is possible that macrophages may represent another cell target for improving corneal neuroregeneration after injury.

Finally, after unilateral corneal injury, what is/are the dominant factor/s that underpin(s) contralateral ocular inflammation and corneal nerve degeneration? Given the growing number of studies reporting contralateral eye effects on corneal nerves and immune cells, this is an important question that could provide more clues into the functional significance of corneal neuroimmune interactions.

With the increased use of IVCN in research and clinical settings, as well as more clinical trials exploring new therapies for corneal neuropathy, it is highly likely that new insights into corneal neuroimmune interactions will be revealed. In addition, continued improvements in techniques for immune cell identification and characterisation *in vivo*, new imaging technologies for visualizing corneal sensory nerve terminals, and robust techniques for

evaluating corneal nerve function will likely be the foundation for gaining a deeper understanding of neuroimmune interactions and developing novel therapeutics for treating corneal neuropathy.

#### Acknowledgements

Funding support provided by the National Health and Medical Research Council (HRC, APP1126540) and the Australian Government Research Training Program (MW). The authors wish to thank Dr. Alexis Ceecee Britten-Jones for providing the IVCM images in Figure 2, and to Dr. Cecilia Naranjo Golborne and Stephanie Spiridonidis for their contributions to data collection. The authors acknowledge the Florey Advanced Microscopy Facility at the Florey Institute of Neuroscience & Mental Health Facility for provision of instrumentation, training and general support, and the Biological Optical Microscopy Platform for assistance with 3D image analysis.

## Figure captions

Figure 1. Schematic illustration of corneal anatomy. SBNP, sub-basal nerve plexus; SNT, superficial nerve terminals. Note: diagram not to scale.

Figure 2. In vivo confocal microscope images of corneal nerves and immune cells in a healthy individual. (A) Normal appearance of the sub-basal nerve plexus that forms a whorl-like pattern in the paracentral cornea. (B) Sub-basal nerve plexus and immune cells in the healthy central cornea. Magnified images on the right panel show so-called ‘immature’ dendritic cells (DCs) without dendrites (red rectangle) and ‘mature’ DCs with dendrites (yellow rectangle, based on the classification by Lagali et al., 2018). Note that the ‘mature’ DC process appears to contact the nerve axon. Scale bar (100  $\mu\text{m}$ ) applies to images A and B. Adapted and modified from Chinnery et al., 2021.

Figure 3. Transgenic mouse models for visualization of corneal nerves and immune cells. (A) Thy1-YFP transgenic mice visualize corneal sensory nerves in vivo. Adapted and modified from Namavari et al., 2011. (B-C) CD11c-YFP  $\times$  thy1-YFP mice label both CD11c<sup>+</sup> DCs and thy1<sup>+</sup> neurons. Scale bars = 100  $\mu\text{m}$ . (D) Cx3cr1<sup>GFP/+</sup> mice label Cx3cr1<sup>+</sup> cells that are resident to corneal stroma and epithelium. MHC-II<sup>+</sup> Cx3cr1<sup>+</sup> cells localized in the epithelium are DCs. (E) Cx3cr1<sup>GFP/GFP</sup> mice label Cx3cr1<sup>+</sup> cells but there is a lack of epithelial DCs due to Cx3cr1-deficiency. (F-G) Wild-type and Cx3cr1-deficient mice show a complete loss of intraepithelial DCs (magenta) in Cx3cr1-deficient mice, but normal presence of stromal macrophages (cyan) (G). Panel B and C adapted and modified from Jamali et al., 2020b. Panel D from Chinnery et al., 2017a.

Figure 4. Corneal intraepithelial nerves in a C57BL/6J mouse. (A) Sub-basal nerve plexus (SBNP) nerves forming a whorl-like pattern in the inferonasal, paracentral cornea. (B) Superficial nerve terminals (SNT) arising from the SBNP and extending towards the epithelial surface. (C) Merged image of the two layers with false-colored projections. Magenta: SBNP, cyan: SNT. Scale bars = 50  $\mu\text{m}$ .

Figure 5. Schematic illustration of neural arc of inflammation in dry eye disease. Abbreviations: TRPV1, transient receptor potential vanilloid 1; TRPM8, transient receptor potential melastatin 8; SP, substance P.

Figure 6. Corneal immune cells in mice during homeostasis and inflammation. (A1-A4) Resident CD45<sup>+</sup>CD11c<sup>+</sup> dendritic cells localized at the epithelial layer in an intact cornea, showing their typical dendriform shape. (B1-B4) Resident CD45<sup>+</sup>Iba1<sup>+</sup> macrophages in the stroma in an intact cornea. (C1-C4) Infiltrating NIMP<sup>+</sup> Cx3cr1<sup>-</sup> neutrophils in the stroma in a sterile injured cornea, characterized with a round cell shape and multi-lobed nucleus. Cx3cr1<sup>+</sup> cells in C2 are macrophages. Scale bar (50  $\mu$ m) in C4 applies to all images.

Figure 7. Corneal neuroimmune interactions based on physical proximity between epithelial DCs and nerves. (A-D) CD45<sup>+</sup> DCs and PGP9.5<sup>+</sup> sensory nerves in the epithelium of a normal mouse cornea. Surface rendering of CD45 and PGP9.5 channels in Imaris reveal the voxels that represent surface overlap (D). No difference in the degree of surface overlap ('neuroimmune interaction') in the central and peripheral cornea (E). After short (i.e. 10 minute) exposure to topical agonists in vivo, the degree of neuroimmune interaction was greater after exposure to TNF- $\alpha$  (F-H). The degree of surface overlap between TRPV1<sup>+</sup>BIII<sup>+</sup> corneal nerves with corneal DCs was higher after sterile corneal injury (I). In humans, IVCN imaging enables visualization and quantitative analysis of 'non-dendritic' cells (NCF) and 'dendritic cells' (DCF) in contact with nerve fibres (J; NCF). The number of DCs in contact with nerve fibres is higher in patients with inflammatory neuropathy. Panel I adapted from Jiao et al., 2021; panel J adapted from Stettner et al., 2016.

Figure 8. Pre-clinical evidence for functional neuroimmune interactions in corneal sterile injury, in wild-type mice (with DCs, arrowheads; A) and Cx3cr1<sup>gfp/gfp</sup> (without DCs; B, C). (D-H) Improved corneal nerve ( $\beta$ III<sup>+</sup>) regeneration following sterile injury was observed after topical decorin application in wild type mice, but not in Cx3cr1<sup>gfp/gfp</sup> mice. Scale bar in A&B = 100  $\mu$ m, scale bar in D-G =50  $\mu$ m.

Figure 9. Schematic illustration of proposed corneal neuroimmune interactions.

Abbreviations: BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; NGF, nerve growth factor; SBNP, sub-basal nerve plexus; SNT, superficial nerve terminals.

### **Supplementary information:**



Supp Figure 1: *En face* flatmount of PGP9.5<sup>+</sup> sensory nerves (A) and CD45<sup>+</sup> stromal macrophages (B) in the healthy central cornea of a mouse. A color-coded depth projection showing the presence of smaller cellular processes (arrows) from CD45<sup>+</sup> stromal cells which are visible in the sub-basal nerve plexus.

Supplementary Video 1: 3D rendered reconstruction of stromal macrophages (purple) located in the anterior stroma of the central mouse cornea. Cellular processes from the stromal macrophages appear to interact with the sub-basal nerve axons which are located in the basal layer of the epithelium.

## References

- Acosta, C.M., Luna, C., Quirce, S., Belmonte, C., Gallar, J., 2013. Changes in sensory activity of ocular surface sensory nerves during allergic keratoconjunctivitis. *Pain* 154, 2353-2362.
- Acosta, M.C., Peral, A., Luna, C., Pintor, J., Belmonte, C., Gallar, J., 2004. Tear secretion induced by selective stimulation of corneal and conjunctival sensory nerve fibers. *Invest Ophthalmol Vis Sci* 45, 2333-2336.
- Aggarwal, S., Cavalcanti, B.M., Regali, L., Cruzat, A., Trinidad, M., Williams, C., Jurkunas, U.V., Hamrah, P., 2018. In Vivo Confocal Microscopy Shows Alterations in Nerve Density and Dendritiform Cell Density in Fuchs' Endothelial Corneal Dystrophy. *Am J Ophthalmol* 196, 136-144.
- Aggarwal, S., Colon, C., Kheirkhah, A., Hamrah, P., 2019. Efficacy of autologous serum tears for treatment of neuropathic corneal pain. *Ocul Surf* 17, 532-539.
- Aggarwal, S., Kheirkhah, A., Cavalcanti, B.M., Cruzat, A., Colon, C., Brown, E., Borsook, D., Pruss, H., Hamrah, P., 2015. Autologous Serum Tears for Treatment of Photoallodynia in Patients with Corneal Neuropathy: Efficacy and Evaluation with In Vivo Confocal Microscopy. *Ocul Surf* 13, 250-262.
- Aggarwal, S., Kheirkhah, A., Cavalcanti, B.M., Cruzat, A., Jamali, A., Hamrah, P., 2020. Correlation of corneal immune cell changes with clinical severity in dry eye disease: An in vivo confocal microscopy study. *Ocul Surf*.
- Aghanoori, M.R., Smith, D.R., Shariati-Ievvari, S., Ajisebutu, A., Nguyen, A., Desmond, F., Jesus, C.H.A., Zhou, X., Calcutt, N.A., Aliani, M., Fernyhough, P., 2019. Insulin-like growth factor-1 activates AMPK to augment mitochondrial function and correct neuronal metabolism in sensory neurons in type 1 diabetes. *Mol Metab* 20, 149-165.
- Agnifili, L., Brescia, L., Villani, E., D'Onofrio, G., Figus, M., Oddone, F., Nucci, P., Mastropasqua, R., 2022. In Vivo Confocal Microscopy of the Corneal Sub-Basal Nerve plexus in Medically Controlled Glaucoma. *Microsc Microanal*, 1-8.
- Ahuja, Y., Baratz, K.H., McLaren, J.W., Bourne, W.M., Patel, S.V., 2012. Decreased corneal sensitivity and abnormal corneal nerves in Fuchs endothelial dystrophy. *Cornea* 31, 1257-1263.
- Al-Aqaba, M., Calienno, R., Fares, U., Otri, A.M., Mastropasqua, L., Nubile, M., Dua, H.S., 2012a. The effect of standard and transepithelial ultraviolet collagen cross-linking on human corneal nerves: an ex vivo study. *Am J Ophthalmol* 153, 258-266 e252.
- Al-Aqaba, M.A., Dhillon, V.K., Mohammed, I., Said, D.G., Dua, H.S., 2019. Corneal nerves in health and disease. *Prog Retin Eye Res* 73, 100762.
- Al-Aqaba, M.A., Fares, U., Suleman, H., Lowe, J., Dua, H.S., 2010. Architecture and distribution of human corneal nerves. *Br J Ophthalmol* 94, 784-789.
- Al-Aqaba, M.A., Otri, A.M., Fares, U., Miri, A., Dua, H.S., 2012b. Organization of the regenerated nerves in human corneal grafts. *Am J Ophthalmol* 153, 29-37 e24.
- Alamri, A., Bron, R., Brock, J.A., Ivanusic, J.J., 2015. Transient receptor potential cation channel subfamily V member 1 expressing corneal sensory neurons can be subdivided into at least three subpopulations. *Front Neuroanat* 9, 71.
- Alamri, A.S., Brock, J.A., Herath, C.B., Rajapaksha, I.G., Angus, P.W., Ivanusic, J.J., 2019. The Effects of Diabetes and High-Fat Diet on Polymodal Nociceptor and Cold Thermoreceptor Nerve Terminal Endings in the Corneal Epithelium. *Invest Ophthalmol Vis Sci* 60, 209-217.
- Amouzegar, A., Chauhan, S.K., Dana, R., 2016. Alloimmunity and Tolerance in Corneal Transplantation. *J Immunol* 196, 3983-3991.
- Ansel, J.C., Brown, J.R., Payan, D.G., Brown, M.A., 1993. Substance P selectively activates TNF-alpha gene expression in murine mast cells. *J Immunol* 150, 4478-4485.
- Arranz-Marquez, E., Katsanos, A., Kozobolis, V.P., Konstas, A.G.P., Teus, M.A., 2019. A Critical Overview of the Biological Effects of Mitomycin C Application on the Cornea Following Refractive Surgery. *Adv Ther* 36, 786-797.
- Ashrafi, M., Baguneid, M., Bayat, A., 2016. The Role of Neuromediators and Innervation in Cutaneous Wound Healing. *Acta Derm Venereol* 96, 587-594.

Badian, R.A., Andreasson, M., Svenningsson, P., Utheim, T.P., Lagali, N., 2021. The pattern of the inferocentral whorl region of the corneal subbasal nerve plexus is altered with age. *Ocul Surf* 22, 204-212.

Bandell, M., Story, G.M., Hwang, S.W., Viswanath, V., Eid, S.R., Petrus, M.J., Earley, T.J., Patapoutian, A., 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 41, 849-857.

Barbariga, M., Fonteyne, P., Ostadreza, M., Bignami, F., Rama, P., Ferrari, G., 2018. Substance P Modulation of Human and Murine Corneal Neovascularization. *Invest Ophthalmol Vis Sci* 59, 1305-1312.

Baron, R., 2006. Mechanisms of disease: neuropathic pain--a clinical perspective. *Nat Clin Pract Neurol* 2, 95-106.

Barros, A., Queiruga-Pineiro, J., Lozano-Sanroma, J., Alcalde, I., Gallar, J., Fernandez-Vega Cueto, L., Alfonso, J.F., Quiros, L.M., Merayo-Llodes, J., 2022. Small fiber neuropathy in the cornea of Covid-19 patients associated with the generation of ocular surface disease. *Ocul Surf* 23, 40-48.

Battat, L., Macri, A., Dursun, D., Pflugfelder, S.C., 2001. Effects of laser in situ keratomileusis on tear production, clearance, and the ocular surface. *Ophthalmology* 108, 1230-1235.

Baudouin, C., Aragona, P., Messmer, E.M., Tomlinson, A., Calonge, M., Boboridis, K.G., Akova, Y.A., Geerling, G., Labetoulle, M., Rolando, M., 2013. Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting. *Ocul Surf* 11, 246-258.

Bech, F., Gonzalez-Gonzalez, O., Artime, E., Serrano, J., Alcalde, I., Gallar, J., Merayo-Llodes, J., Belmonte, C., 2018. Functional and Morphologic Alterations in Mechanical, Polymodal, and Cold Sensory Nerve Fibers of the Cornea Following Photorefractive Keratectomy. *Invest Ophthalmol Vis Sci* 59, 2281-2292.

Belmonte, C., Acosta, M.C., Gallar, J., 2004. Neural basis of sensation in intact and injured corneas. *Exp Eye Res* 78, 513-525.

Belmonte, C., Gallar, J., 2011. Cold thermoreceptors, unexpected players in tear production and ocular dryness sensations. *Invest Ophthalmol Vis Sci* 52, 3888-3892.

Belmonte, C., Luna, C.L., Gallar, J., 2003. Cgrp is Released by Selective Stimulation of Polymodal Nociceptor but Not of Cold Receptor Nerve Fibers of the Cornea. *Investigative Ophthalmology & Visual Science* 44, 1391-1391.

Belmonte, C., Nichols, J.J., Cox, S.M., Brock, J.A., Begley, C.G., Bereiter, D.A., Dartt, D.A., Galor, A., Hamrah, P., Ivanusic, J.J., Jacobs, D.S., McNamara, N.A., Rosenblatt, M.I., Stapleton, F., Wolffsohn, J.S., 2017. TFOS DEWS II pain and sensation report. *Ocul Surf* 15, 404-437.

Beuerman, R.W., Schimmelpfennig, B., 1980. Sensory denervation of the rabbit cornea affects epithelial properties. *Exp Neurol* 69, 196-201.

Bignami, F., Giacomini, C., Lorusso, A., Aramini, A., Rama, P., Ferrari, G., 2014. NK1 receptor antagonists as a new treatment for corneal neovascularization. *Invest Ophthalmol Vis Sci* 55, 6783-6794.

Bignami, F., Rama, P., Ferrari, G., 2016. Substance P and its Inhibition in Ocular Inflammation. *Curr Drug Targets* 17, 1265-1274.

Bikbova, G., Oshitari, T., Baba, T., Bikbov, M., Yamamoto, S., 2018. Diabetic corneal neuropathy: clinical perspectives. *Clin Ophthalmol* 12, 981-987.

Binshtok, A.M., Wang, H., Zimmermann, K., Amaya, F., Vardeh, D., Shi, L., Brenner, G.J., Ji, R.R., Bean, B.P., Woolf, C.J., Samad, T.A., 2008. Nociceptors are interleukin-1beta sensors. *J Neurosci* 28, 14062-14073.

Bitirgen, G., Akpinar, Z., Malik, R.A., Ozkagnici, A., 2017. Use of Corneal Confocal Microscopy to Detect Corneal Nerve Loss and Increased Dendritic Cells in Patients With Multiple Sclerosis. *JAMA Ophthalmol* 135, 777-782.

Bitirgen, G., Korkmaz, C., Zamani, A., Ozkagnici, A., Zengin, N., Ponirakis, G., Malik, R.A., 2021. Corneal confocal microscopy identifies corneal nerve fibre loss and increased dendritic cells in patients with long COVID. *Br J Ophthalmol*.

Bitirgen, G., Tinkir Kayitmazbatir, E., Satirtav, G., Malik, R.A., Ozkagnici, A., 2018a. In Vivo Confocal Microscopic Evaluation of Corneal Nerve Fibers and Dendritic Cells in Patients With Behcet's Disease. *Front Neurol* 9, 204.

Bitirgen, G., Turkmen, K., Malik, R.A., Ozkagnici, A., Zengin, N., 2018b. Corneal confocal microscopy detects corneal nerve damage and increased dendritic cells in Fabry disease. *Sci Rep* 8, 12244.

Blake, K.J., Jiang, X.R., Chiu, I.M., 2019. Neuronal Regulation of Immunity in the Skin and Lungs. *Trends Neurosci* 42, 537-551.

Bonini, S., Lambiase, A., Rama, P., Caprioglio, G., Aloe, L., 2000. Topical treatment with nerve growth factor for neurotrophic keratitis. *Ophthalmology* 107, 1347-1351; discussion 1351-1342.

Bonini, S., Lambiase, A., Rama, P., Sinigaglia, F., Allegretti, M., Chao, W., Mantelli, F., Group, R.S., 2018. Phase II Randomized, Double-Masked, Vehicle-Controlled Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis. *Ophthalmology* 125, 1332-1343.

Bouheraoua, N., Jouve, L., El Sanharawi, M., Sandali, O., Temstet, C., Loriaut, P., Basli, E., Borderie, V., Laroche, L., 2014. Optical coherence tomography and confocal microscopy following three different protocols of corneal collagen-crosslinking in keratoconus. *Invest Ophthalmol Vis Sci* 55, 7601-7609.

Brash, J.T., Denti, L., Ruhrberg, C., Bucher, F., 2019. VEGF188 promotes corneal reinnervation after injury. *JCI Insight* 4.

Brissette-Storkus, C.S., Reynolds, S.M., Lepisto, A.J., Hendricks, R.L., 2002. Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest Ophthalmol Vis Sci* 43, 2264-2271.

Britten-Jones, A.C., Kamel, J.T., Roberts, L.J., Braat, S., Craig, J.P., MacIsaac, R.J., Downie, L.E., 2021. Investigating the Neuroprotective Effect of Oral Omega-3 Fatty Acid Supplementation in Type 1 Diabetes (nPROOFS1): A Randomized Placebo-Controlled Trial. *Diabetes* 70, 1794-1806.

Bron, A.J., de Paiva, C.S., Chauhan, S.K., Bonini, S., Gabison, E.E., Jain, S., Knop, E., Markoulli, M., Ogawa, Y., Perez, V., Uchino, Y., Yokoi, N., Zoukhri, D., Sullivan, D.A., 2017. TFOS DEWS II pathophysiology report. *Ocul Surf* 15, 438-510.

Brown, B.N., Ratner, B.D., Goodman, S.B., Amar, S., Badylak, S.F., 2012. Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. *Biomaterials* 33, 3792-3802.

Bucher, F., Hos, D., Matthaehi, M., Steven, P., Cursiefen, C., Heindl, L.M., 2014. Corneal nerve alterations after Descemet membrane endothelial keratoplasty: an in vivo confocal microscopy study. *Cornea* 33, 1134-1139.

Bursch, L.S., Wang, L., Igyarto, B., Kissenpfennig, A., Malissen, B., Kaplan, D.H., Hogquist, K.A., 2007. Identification of a novel population of Langerin+ dendritic cells. *J Exp Med* 204, 3147-3156.

Calder, P.C., 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans* 45, 1105-1115.

Callejo, G., Castellanos, A., Castany, M., Gual, A., Luna, C., Acosta, M.C., Gallar, J., Giblin, J.P., Gasull, X., 2015. Acid-sensing ion channels detect moderate acidifications to induce ocular pain. *Pain* 156, 483-495.

Calvillo, M.P., McLaren, J.W., Hodge, D.O., Bourne, W.M., 2004. Corneal reinnervation after LASIK: prospective 3-year longitudinal study. *Invest Ophthalmol Vis Sci* 45, 3991-3996.

Campagnolo, M., Lazzarini, D., Fregona, I., Cacciavillani, M., Bergamo, F., Parrozzani, R., Midena, E., Briani, C., 2013. Corneal confocal microscopy in patients with oxaliplatin-induced peripheral neuropathy. *J Peripher Nerv Syst* 18, 269-271.

Caporossi, A., Mazzotta, C., Baiocchi, S., Caporossi, T., Paradiso, A.L., 2012. Transepithelial corneal collagen crosslinking for keratoconus: qualitative investigation by in vivo HRT II confocal analysis. *Eur J Ophthalmol* 22 Suppl 7, S81-88.

Carlson, E.C., Drazba, J., Yang, X., Perez, V.L., 2006. Visualization and characterization of inflammatory cell recruitment and migration through the corneal stroma in endotoxin-induced keratitis. *Invest Ophthalmol Vis Sci* 47, 241-248.

Cavalcanti, B.M., Cruzat, A., Sahin, A., Pavan-Langston, D., Samayoa, E., Hamrah, P., 2018. In vivo confocal microscopy detects bilateral changes of corneal immune cells and nerves in unilateral herpes zoster ophthalmicus. *Ocul Surf* 16, 101-111.

Chaudhary, S., Namavari, A., Yco, L., Chang, J.H., Sonawane, S., Khanolkar, V., Sarkar, J., Jain, S., 2012. Neurotrophins and nerve regeneration-associated genes are expressed in the cornea after lamellar flap surgery. *Cornea* 31, 1460-1467.

Chen, P., Bonaldo, P., 2013. Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. *Int Rev Cell Mol Biol* 301, 1-35.

Chen, P., Piao, X., Bonaldo, P., 2015. Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol* 130, 605-618.

Chen, Z.L., Yu, W.M., Strickland, S., 2007. Peripheral regeneration. *Annu Rev Neurosci* 30, 209-233.

Chinnery, H.R., Humphries, T., Clare, A., Dixon, A.E., Howes, K., Moran, C.B., Scott, D., Zakrzewski, M., Pearlman, E., McMenemy, P.G., 2008. Turnover of bone marrow-derived cells in the irradiated mouse cornea. *Immunology* 125, 541-548.

Chinnery, H.R., Leong, C.M., Chen, W., Forrester, J.V., McMenemy, P.G., 2015. TLR9 and TLR7/8 activation induces formation of keratic precipitates and giant macrophages in the mouse cornea. *J Leukoc Biol* 97, 103-110.

Chinnery, H.R., McMenemy, P.G., Dando, S.J., 2017a. Macrophage physiology in the eye. *Pflugers Arch* 469, 501-515.

Chinnery, H.R., Naranjo Golborne, C., Downie, L.E., 2017b. Omega-3 supplementation is neuroprotective to corneal nerves in dry eye disease: a pilot study. *Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians (Optometrists)* 37, 473-481.

Chinnery, H.R., Rajan, R., Jiao, H., Wu, M., Zhang, A.C., De Silva, M.E.H., Makrai, E., Stepp, M.A., Di Girolamo, N., Downie, L.E., 2021a. Identification of presumed corneal neuromas and microneuromas using laser-scanning in vivo confocal microscopy: a systematic review. *Br J Ophthalmol*.

Chinnery, H.R., Ruitenbergh, M.J., Plant, G.W., Pearlman, E., Jung, S., McMenemy, P.G., 2007. The chemokine receptor CX3CR1 mediates homing of MHC class II-positive cells to the normal mouse corneal epithelium. *Invest Ophthalmol Vis Sci* 48, 1568-1574.

Chinnery, H.R., Zhang, X.Y., Wu, C.Y., Downie, L.E., 2021b. Corneal immune cell morphometry as an indicator of local and systemic pathology: A review. *Clin Exp Ophthalmol* 49, 729-740.

Choi, E.Y., Kang, H.G., Lee, C.H., Yeo, A., Noh, H.M., Gu, N., Kim, M.J., Song, J.S., Kim, H.C., Lee, H.K., 2017. Langerhans cells prevent subbasal nerve damage and upregulate neurotrophic factors in dry eye disease. *PLoS One* 12, e0176153.

Chucair-Elliott, A.J., Gurung, H.R., Carr, M.M., Carr, D.J.J., 2017. Colony Stimulating Factor-1 Receptor Expressing Cells Infiltrating the Cornea Control Corneal Nerve Degeneration in Response to HSV-1 Infection. *Invest Ophthalmol Vis Sci* 58, 4670-4682.

Chucair-Elliott, A.J., Jenkins, J., Carr, M.M., Carr, D.J., 2016. IL-6 Contributes to Corneal Nerve Degeneration after Herpes Simplex Virus Type I Infection. *Am J Pathol* 186, 2665-2678.

Chucair-Elliott, A.J., Zheng, M., Carr, D.J., 2015. Degeneration and regeneration of corneal nerves in response to HSV-1 infection. *Invest Ophthalmol Vis Sci* 56, 1097-1107.

Chung, E.S., Lee, K.H., Kim, M., Chang, E.J., Chung, T.Y., Kim, E.K., Lee, H.K., 2013. Expression of neurotrophic factors and their receptors in keratoconic cornea. *Curr Eye Res* 38, 743-750.

Colorado, L.H., Markoulli, M., Edwards, K., 2019. The Relationship Between Corneal Dendritic Cells, Corneal Nerve Morphology and Tear Inflammatory Mediators and Neuropeptides in Healthy Individuals. *Curr Eye Res* 44, 840-848.

Coppey, L., Davidson, E., Shevalye, H., Obrosova, A., Torres, M., Yorek, M.A., 2020. Progressive Loss of Corneal Nerve Fibers and Sensitivity in Rats Modeling Obesity and Type 2 Diabetes Is Reversible with Omega-3 Fatty Acid Intervention: Supporting Cornea Analyses as a Marker for Peripheral Neuropathy and Treatment. *Diabetes Metab Syndr Obes* 13, 1367-1384.

Cortina, M.S., He, J., Li, N., Bazan, N.G., Bazan, H.E., 2012. Recovery of corneal sensitivity, calcitonin gene-related peptide-positive nerves, and increased wound healing induced by pigment epithelial-derived factor plus docosahexaenoic acid after experimental surgery. *Arch Ophthalmol* 130, 76-83.

Cortina, M.S., He, J., Russ, T., Bazan, N.G., Bazan, H.E., 2013. Neuroprotectin D1 restores corneal nerve integrity and function after damage from experimental surgery. *Invest Ophthalmol Vis Sci* 54, 4109-4116.

Courson, J.A., Smith, I., Do, T., Landry, P.T., Hargrave, A., Behzad, A.R., Hanlon, S.D., Rumbaut, R.E., Smith, C.W., Burns, A.R., 2019. Serial block-face scanning electron microscopy reveals neuronal-epithelial cell fusion in the mouse cornea. *PLoS One* 14, e0224434.

Cox, S.M., Kheirkhah, A., Aggarwal, S., Abedi, F., Cavalcanti, B.M., Cruzat, A., Hamrah, P., 2021. Alterations in corneal nerves in different subtypes of dry eye disease: An in vivo confocal microscopy study. *Ocul Surf* 22, 135-142.

Craig, J.P., Nichols, K.K., Akpek, E.K., Caffery, B., Dua, H.S., Joo, C.K., Liu, Z., Nelson, J.D., Nichols, J.J., Tsubota, K., Stapleton, F., 2017. TFOS DEWS II Definition and Classification Report. *Ocul Surf* 15, 276-283.

Cruzat, A., Qazi, Y., Hamrah, P., 2017. In Vivo Confocal Microscopy of Corneal Nerves in Health and Disease. *Ocul Surf* 15, 15-47.

Cruzat, A., Schrems, W.A., Schrems-Hoesl, L.M., Cavalcanti, B.M., Baniyadi, N., Witkin, D., Pavan-Langston, D., Dana, R., Hamrah, P., 2015. Contralateral Clinically Unaffected Eyes of Patients With Unilateral Infectious Keratitis Demonstrate a Sympathetic Immune Response. *Invest Ophthalmol Vis Sci* 56, 6612-6620.

Cruzat, A., Witkin, D., Baniyadi, N., Zheng, L., Ciolino, J.B., Jurkunas, U.V., Chodosh, J., Pavan-Langston, D., Dana, R., Hamrah, P., 2011. Inflammation and the nervous system: the connection in the cornea in patients with infectious keratitis. *Invest Ophthalmol Vis Sci* 52, 5136-5143.

Cunin, P., Caillon, A., Corvaisier, M., Garo, E., Scotet, M., Blanchard, S., Delneste, Y., Jeannin, P., 2011. The tachykinins substance P and hemokinin-1 favor the generation of human memory Th17 cells by inducing IL-1beta, IL-23, and TNF-like 1A expression by monocytes. *J Immunol* 186, 4175-4182.

D'Onofrio, L., Kalteniece, A., Ferdousi, M., Azmi, S., Petropoulos, I.N., Ponirakis, G., Alam, U., Asghar, O., Marshall, A., Boulton, A.J.M., Efron, N., Buzzetti, R., Soran, H., Malik, R.A., 2021. Small Nerve Fiber Damage and Langerhans Cells in Type 1 and Type 2 Diabetes and LADA Measured by Corneal Confocal Microscopy. *Invest Ophthalmol Vis Sci* 62, 5.

Darwish, T., Brahma, A., Efron, N., O'Donnell, C., 2007. Subbasal nerve regeneration after penetrating keratoplasty. *Cornea* 26, 935-940.

Davies, S.J., Goucher, D.R., Doller, C., Silver, J., 1999. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. *J Neurosci* 19, 5810-5822.

De Cilla, S., Fogagnolo, P., Sacchi, M., Orzalesi, N., Carini, E., Ceresara, G., Rossetti, L., 2014. Corneal involvement in uneventful cataract surgery: an in vivo confocal microscopy study. *Ophthalmologica* 231, 103-110.

De Paiva, C.S., Chen, Z., Koch, D.D., Hamill, M.B., Manuel, F.K., Hassan, S.S., Wilhelmus, K.R., Pflugfelder, S.C., 2006. The incidence and risk factors for developing dry eye after myopic LASIK. *Am J Ophthalmol* 141, 438-445.

De Silva, M.E.H., Hill, L.J., Downie, L.E., Chinnery, H.R., 2019. The Effects of Aging on Corneal and Ocular Surface Homeostasis in Mice. *Invest Ophthalmol Vis Sci* 60, 2705-2715.

De Silva, M.E.H., Zhang, A.C., Karahalios, A., Chinnery, H.R., Downie, L.E., 2017. Laser scanning in vivo confocal microscopy (IVCM) for evaluating human corneal sub-basal nerve plexus parameters: protocol for a systematic review. *BMJ Open* 7, e018646.

Dehghani, C., Frost, S., Jayasena, R., Fowler, C., Masters, C.L., Kanagasingam, Y., Jiao, H., Lim, J.K.H., Chinnery, H.R., Downie, L.E., 2020. Morphometric Changes to Corneal Dendritic Cells in Individuals With Mild Cognitive Impairment. *Front Neurosci* 14, 556137.

Deinema, L.A., Vingrys, A.J., Wong, C.Y., Jackson, D.C., Chinnery, H.R., Downie, L.E., 2017. A Randomized, Double-Masked, Placebo-Controlled Clinical Trial of Two Forms of Omega-3 Supplements for Treating Dry Eye Disease. *Ophthalmology* 124, 43-52.

Delic, N.C., Cai, J.R., Watson, S.L., Downie, L.E., Di Girolamo, N., 2022. Evaluating the clinical translational relevance of animal models for limbal stem cell deficiency: A systematic review. *Ocul Surf* 23, 169-183.

Denoyer, A., Landman, E., Trinh, L., Faure, J.F., Auclin, F., Baudouin, C., 2015. Dry eye disease after refractive surgery: comparative outcomes of small incision lenticule extraction versus LASIK. *Ophthalmology* 122, 669-676.

Di, G., Qi, X., Zhao, X., Zhang, S., Danielson, P., Zhou, Q., 2017a. Corneal Epithelium-Derived Neurotrophic Factors Promote Nerve Regeneration. *Invest Ophthalmol Vis Sci* 58, 4695-4702.

Di, G., Zhao, X., Qi, X., Zhang, S., Feng, L., Shi, W., Zhou, Q., 2017b. VEGF-B promotes recovery of corneal innervations and trophic functions in diabetic mice. *Sci Rep* 7, 40582.

Ding, W., Stohl, L.L., Wagner, J.A., Granstein, R.D., 2008. Calcitonin gene-related peptide biases Langerhans cells toward Th2-type immunity. *J Immunol* 181, 6020-6026.

Doss, A.L., Smith, P.G., 2014. Langerhans cells regulate cutaneous innervation density and mechanical sensitivity in mouse footpad. *Neurosci Lett* 578, 55-60.

Downie, L.E., Bandlitz, S., Bergmanson, J.P.G., Craig, J.P., Dutta, D., Maldonado-Codina, C., Ngo, W., Siddireddy, J.S., Wolffsohn, J.S., 2021. CLEAR - Anatomy and physiology of the anterior eye. *Cont Lens Anterior Eye* 44, 132-156.

Downie, L.E., Gad, A., Wong, C.Y., Gray, J.H.V., Zeng, W., Jackson, D.C., Vingrys, A.J., 2018. Modulating Contact Lens Discomfort With Anti-Inflammatory Approaches: A Randomized Controlled Trial. *Invest Ophthalmol Vis Sci* 59, 3755-3766.

Downie, L.E., Ng, S.M., Lindsley, K.B., Akpek, E.K., 2019. Omega-3 and omega-6 polyunsaturated fatty acids for dry eye disease. *Cochrane Database Syst Rev* 12, CD011016.

Dua, H.S., Said, D.G., Messmer, E.M., Rolando, M., Benitez-Del-Castillo, J.M., Hossain, P.N., Shortt, A.J., Geerling, G., Nubile, M., Figueiredo, F.C., Rauz, S., Mastropasqua, L., Rama, P., Baudouin, C., 2018. Neurotrophic keratopathy. *Prog Retin Eye Res* 66, 107-131.

Dubovy, P., 2011. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. *Ann Anat* 193, 267-275.

Ehinger, B., 1966. Connections between adrenergic nerves and other tissue components in the eye. *Acta Physiol Scand* 67, 57-64.

Ekstrand, A.J., Cao, R., Bjorndahl, M., Nystrom, S., Jonsson-Rylander, A.C., Hassani, H., Hallberg, B., Nordlander, M., Cao, Y., 2003. Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. *Proc Natl Acad Sci U S A* 100, 6033-6038.

Erie, J.C., McLaren, J.W., Hodge, D.O., Bourne, W.M., 2005. Recovery of corneal subbasal nerve density after PRK and LASIK. *Am J Ophthalmol* 140, 1059-1064.

Erkan Turan, K., Kocabeyoglu, S., Unal-Cevik, I., Bezci, F., Akinci, A., Irkec, M., 2018. Ocular Surface Alterations in the Context of Corneal In Vivo Confocal Microscopic Characteristics in Patients With Fibromyalgia. *Cornea* 37, 205-210.

Esquenazi, S., Bazan, H.E., Bui, V., He, J., Kim, D.B., Bazan, N.G., 2005. Topical combination of NGF and DHA increases rabbit corneal nerve regeneration after photorefractive keratectomy. *Invest Ophthalmol Vis Sci* 46, 3121-3127.

Fakih, D., Zhao, Z., Nicolle, P., Reboussin, E., Joubert, F., Luzu, J., Labbe, A., Rostene, W., Baudouin, C., Melik Parsadaniantz, S., Reaux-Le Goazigo, A., 2019. Chronic dry eye induced corneal hypersensitivity, neuroinflammatory responses, and synaptic plasticity in the mouse trigeminal brainstem. *J Neuroinflammation* 16, 268.

Farhangi, M., Diel, R.J., Buse, D.C., Huang, A.M., Levitt, R.C., Sarantopoulos, C.D., Felix, E.R., Galor, A., 2020. Individuals with migraine have a different dry eye symptom profile than individuals without migraine. *Br J Ophthalmol* 104, 260-264.

Feng, P., Yee, K.K., Rawson, N.E., Feldman, L.M., Feldman, R.S., Breslin, P.A., 2009. Immune cells of the human peripheral taste system: dominant dendritic cells and CD4 T cells. *Brain Behav Immun* 23, 760-766.

Ferdousi, M., Romanchuk, K., Mah, J.K., Virtanen, H., Millar, C., Malik, R.A., Pacaud, D., 2019. Early corneal nerve fibre damage and increased Langerhans cell density in children with type 1 diabetes mellitus. *Sci Rep* 9, 8758.

Ferrari, G., Nallasamy, N., Downs, H., Dana, R., Oaklander, A.L., 2013. Corneal innervation as a window to peripheral neuropathies. *Exp Eye Res* 113, 148-150.

Fini, M.E., Girard, M.T., Matsubara, M., 1992. Collagenolytic/gelatinolytic enzymes in corneal wound healing. *Acta Ophthalmol Suppl*, 26-33.

Fogagnolo, P., Dipinto, A., Vanzulli, E., Maggiolo, E., De Cilla, S., Autelitano, A., Rossetti, L., 2015. A 1-year randomized study of the clinical and confocal effects of tafluprost and latanoprost in newly diagnosed glaucoma patients. *Adv Ther* 32, 356-369.

Foldenauer, M.E., McClellan, S.A., Barrett, R.P., Zhang, Y., Hazlett, L.D., 2012. Substance P affects growth factors in *Pseudomonas aeruginosa*-infected mouse cornea. *Cornea* 31, 1176-1188.

Frank, G.M., Buella, K.A., Maker, D.M., Harvey, S.A., Hendricks, R.L., 2012. Early responding dendritic cells direct the local NK response to control herpes simplex virus 1 infection within the cornea. *J Immunol* 188, 1350-1359.

Fujishima, H., Takeyama, M., Takeuchi, T., Saito, I., Tsubota, K., 1997. Elevated levels of substance P in tears of patients with allergic conjunctivitis and vernal keratoconjunctivitis. *Clin Exp Allergy* 27, 372-378.

Gad, A., Vingrys, A.J., Wong, C.Y., Jackson, D.C., Downie, L.E., 2019. Tear film inflammatory cytokine upregulation in contact lens discomfort. *Ocul Surf* 17, 89-97.

Gao, N., Lee, P., Yu, F.S., 2016a. Intraepithelial dendritic cells and sensory nerves are structurally associated and functional interdependent in the cornea. *Scientific reports* 6, 36414.

Gao, N., Yan, C., Lee, P., Sun, H., Yu, F.S., 2016b. Dendritic cell dysfunction and diabetic sensory neuropathy in the cornea. *J Clin Invest* 126, 1998-2011.

Gao, N., Yin, J., Yoon, G.S., Mi, Q.S., Yu, F.S., 2011. Dendritic cell-epithelium interplay is a determinant factor for corneal epithelial wound repair. *Am J Pathol* 179, 2243-2253.

Gaudet, A.D., Popovich, P.G., Ramer, M.S., 2011. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8, 110.

Giannaccare, G., Bernabei, F., Pellegrini, M., Guaraldi, F., Turchi, F., Torrazza, C., Senni, C., Scotto, R., Sindaco, D., Di Cello, L., Versura, P., Scordia, V., Traverso, C.E., Vagge, A., 2020. Bilateral morphometric analysis of corneal sub-basal nerve plexus in patients undergoing unilateral cataract surgery: a preliminary in vivo confocal microscopy study. *Br J Ophthalmol*.

Giannaccare, G., Pellegrini, M., Sebastiani, S., Moscardelli, F., Versura, P., Campos, E.C., 2019. In vivo confocal microscopy morphometric analysis of corneal subbasal nerve plexus in dry eye disease using newly developed fully automated system. *Graefes Arch Clin Exp Ophthalmol* 257, 583-589.

Gillette, T.E., Chandler, J.W., Greiner, J.V., 1982. Langerhans cells of the ocular surface. *Ophthalmology* 89, 700-711.

Golebiowski, B., Chao, C., Bui, K.A., Lam, W.Y.W., Richdale, K., Stapleton, F., 2020. Effect of age and contact lens wear on corneal epithelial dendritic cell distribution, density, and morphology. *Cont Lens Anterior Eye* 43, 84-90.

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M.F., Geissmann, F., Rodewald, H.R., 2015. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* 518, 547-551.

Gong, Y., Koh, D.R., 2010. Neutrophils promote inflammatory angiogenesis via release of preformed VEGF in an in vivo corneal model. *Cell Tissue Res* 339, 437-448.

Gordon, S., Martinez-Pomares, L., 2017. Physiological roles of macrophages. *Pflugers Arch* 469, 365-374.



Gordon, S., Taylor, P.R., 2005. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 5, 953-964.

Guzman, M., Miglio, M.S., Zgajnar, N.R., Colado, A., Almejun, M.B., Keitelman, I.A., Sabbione, F., Fuentes, F., Trevani, A.S., Giordano, M.N., Galletti, J.G., 2018. The mucosal surfaces of both eyes are immunologically linked by a neurogenic inflammatory reflex involving TRPV1 and substance P. *Mucosal Immunol* 11, 1441-1453.

Hamrah, P., Cruzat, A., Dastjerdi, M.H., Zheng, L., Shahatit, B.M., Bayhan, H.A., Dana, R., Pavan-Langston, D., 2010. Corneal sensation and subbasal nerve alterations in patients with herpes simplex keratitis: an in vivo confocal microscopy study. *Ophthalmology* 117, 1930-1936.

Hamrah, P., Huq, S.O., Liu, Y., Zhang, Q., Dana, M.R., 2003a. Corneal immunity is mediated by heterogeneous population of antigen-presenting cells. *J Leukoc Biol* 74, 172-178.

Hamrah, P., Liu, Y., Zhang, Q., Dana, M.R., 2003b. Alterations in corneal stromal dendritic cell phenotype and distribution in inflammation. *Arch Ophthalmol* 121, 1132-1140.

Hamrah, P., Liu, Y., Zhang, Q., Dana, M.R., 2003c. The corneal stroma is endowed with a significant number of resident dendritic cells. *Invest Ophthalmol Vis Sci* 44, 581-589.

Hamrah, P., Seyed-Razavi, Y., Yamaguchi, T., 2016. Translational Immunoimaging and Neuroimaging Demonstrate Corneal Neuroimmune Crosstalk. *Cornea* 35 Suppl 1, S20-s24.

Hamrah, P., Zhang, Q., Liu, Y., Dana, M.R., 2002. Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. *Invest Ophthalmol Vis Sci* 43, 639-646.

Hanack, C., Moroni, M., Lima, W.C., Wende, H., Kirchner, M., Adelfinger, L., Schrenk-Siemens, K., Tappe-Theodor, A., Wetzels, C., Kuich, P.H., Gassmann, M., Roggenkamp, D., Bettler, B., Lewin, G.R., Selbach, M., Siemens, J., 2015. GABA blocks pathological but not acute TRPV1 pain signals. *Cell* 160, 759-770.

Hanlon, S.D., Smith, C.W., Sauter, M.N., Burns, A.R., 2014. Integrin-dependent neutrophil migration in the injured mouse cornea. *Exp Eye Res* 120, 61-70.

Hargrave, A., Courson, J.A., Pham, V., Landry, P., Magadi, S., Shankar, P., Hanlon, S., Das, A., Rumbaut, R.E., Smith, C.W., Burns, A.R., 2020. Corneal dysfunction precedes the onset of hyperglycemia in a mouse model of diet-induced obesity. *PLoS One* 15, e0238750.

Harris, D.L., Yamaguchi, T., Hamrah, P., 2018. A Novel Murine Model of Radiation Keratopathy. *Invest Ophthalmol Vis Sci* 59, 3889-3896.

Hatcher, J.B., Soifer, M., Morales, N.G., Farooq, A.V., Perez, V.L., Shieh, C., 2021. Aftermarket effects of cenegermin for neurotrophic keratopathy in pediatric patients. *Ocul Surf* 21, 52-57.

Hattori, T., Chauhan, S.K., Lee, H., Ueno, H., Dana, R., Kaplan, D.H., Saban, D.R., 2011. Characterization of Langerin-expressing dendritic cell subsets in the normal cornea. *Invest Ophthalmol Vis Sci* 52, 4598-4604.

Hazlett, L.D., McClellan, S.A., Barrett, R.P., Liu, J., Zhang, Y., Lighvani, S., 2007. Spantide I decreases type I cytokines, enhances IL-10, and reduces corneal perforation in susceptible mice after *Pseudomonas aeruginosa* infection. *Invest Ophthalmol Vis Sci* 48, 797-807.

He, J., Bazan, H.E., 2016. Neuroanatomy and Neurochemistry of Mouse Cornea. *Invest Ophthalmol Vis Sci* 57, 664-674.

He, J., Cosby, R., Hill, J.M., Bazan, H.E., 2017a. Changes in Corneal Innervation after HSV-1 Latency Established with Different Reactivation Phenotypes. *Curr Eye Res* 42, 181-186.

He, J., Neumann, D., Kakazu, A., Pham, T.L., Musarrat, F., Cortina, M.S., Bazan, H.E.P., 2017b. PEDF plus DHA modulate inflammation and stimulate nerve regeneration after HSV-1 infection. *Exp Eye Res* 161, 153-162.

He, J., Pham, T.L., Bazan, H.E.P., 2021. Neuroanatomy and neurochemistry of rat cornea: Changes with age. *Ocul Surf* 20, 86-94.

He, J., Pham, T.L., Kakazu, A., Bazan, H.E.P., 2017c. Recovery of Corneal Sensitivity and Increase in Nerve Density and Wound Healing in Diabetic Mice After PEDF Plus DHA Treatment. *Diabetes* 66, 2511-2520.

Hindman, H.B., DeMagistris, M., Callan, C., McDaniel, T., Bubel, T., Huxlin, K.R., 2019. Impact of topical anti-fibrotics on corneal nerve regeneration in vivo. *Exp Eye Res* 181, 49-60.

Hori, J., Yamaguchi, T., Keino, H., Hamrah, P., Maruyama, K., 2019. Immune privilege in corneal transplantation. *Prog Retin Eye Res* 72, 100758.

Hosal, B.M., Ornek, N., Zilelioglu, G., Elhan, A.H., 2005. Morphology of corneal nerves and corneal sensation in dry eye: a preliminary study. *Eye (Lond)* 19, 1276-1279.

Hu, K., Harris, D.L., Yamaguchi, T., von Andrian, U.H., Hamrah, P., 2015. A Dual Role for Corneal Dendritic Cells in Herpes Simplex Keratitis: Local Suppression of Corneal Damage and Promotion of Systemic Viral Dissemination. *PLoS One* 10, e0137123.

Huang, C.T., Chu, H.S., Hung, K.C., Chen, L.W., Chen, M.Y., Hu, F.R., Chen, W.L., 2021. The effect of human platelet lysate on corneal nerve regeneration. *Br J Ophthalmol* 105, 884-890.

Ishibashi, F., Okino, M., Ishibashi, M., Kawasaki, A., Endo, N., Kosaka, A., Uetake, H., 2012. Corneal nerve fiber pathology in Japanese type 1 diabetic patients and its correlation with antecedent glycemic control and blood pressure. *J Diabetes Investig* 3, 191-198.

Italiani, P., Boraschi, D., 2014. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol* 5, 514.

Jackson, D.C., Zeng, W., Wong, C.Y., Mifsud, E.J., Williamson, N.A., Ang, C.S., Vingrys, A.J., Downie, L.E., 2016. Tear Interferon-Gamma as a Biomarker for Evaporative Dry Eye Disease. *Invest Ophthalmol Vis Sci* 57, 4824-4830.

Jamali, A., Hu, K., Sendra, V.G., Blanco, T., Lopez, M.J., Ortiz, G., Qazi, Y., Zheng, L., Turhan, A., Harris, D.L., Hamrah, P., 2020a. Characterization of Resident Corneal Plasmacytoid Dendritic Cells and Their Pivotal Role in Herpes Simplex Keratitis. *Cell Rep* 32, 108099.

Jamali, A., Seyed-Razavi, Y., Chao, C., Ortiz, G., Kenyon, B., Blanco, T., Harris, D.L., Hamrah, P., 2020b. Intravital Multiphoton Microscopy of the Ocular Surface: Alterations in Conventional Dendritic Cell Morphology and Kinetics in Dry Eye Disease. *Frontiers in immunology* 11, 742.

Janelins, B.M., Mathers, A.R., Tkacheva, O.A., Erdos, G., Shufesky, W.J., Morelli, A.E., Larregina, A.T., 2009. Proinflammatory tachykinins that signal through the neurokinin 1 receptor promote survival of dendritic cells and potent cellular immunity. *Blood* 113, 3017-3026.

Jeon, K.I., Hindman, H.B., Bubel, T., McDaniel, T., DeMagistris, M., Callan, C., Huxlin, K.R., 2018. Corneal myofibroblasts inhibit regenerating nerves during wound healing. *Scientific reports* 8, 12945.

Jiang, M.S., Yuan, Y., Gu, Z.X., Zhuang, S.L., 2016. Corneal confocal microscopy for assessment of diabetic peripheral neuropathy: a meta-analysis. *Br J Ophthalmol* 100, 9-14.

Jiao, H., Downie, L.E., Huang, X., Wu, M., Oberrauch, S., Keenan, R.J., Jacobson, L.H., Chinnery, H.R., 2020a. Novel alterations in corneal neuroimmune phenotypes in mice with central nervous system tauopathy. *J Neuroinflammation* 17, 136.

Jiao, H., Ivanusic, J.J., McMenamin, P.G., Chinnery, H.R., 2021. Distribution of Corneal TRPV1 and Its Association With Immune Cells During Homeostasis and Injury. *Invest Ophthalmol Vis Sci* 62, 6.

Jiao, H., Lim, A.S., Fazio Coles, T.E., McQuade, R.M., Furness, J.B., Chinnery, H.R., 2020b. The effect of high-fat diet-induced metabolic disturbance on corneal neuroimmune features. *Exp Eye Res* 201, 108298.

Jiao, H., Naranjo Golborne, C., Dando, S.J., McMenamin, P.G., Downie, L.E., Chinnery, H.R., 2019. Topographical and Morphological Differences of Corneal Dendritic Cells during Steady State and Inflammation. *Ocul Immunol Inflamm*, 1-10.

Jing, D., Liu, Z., Chou, Y., Wang, H., Gao, S., Fan, X., Wang, W., 2021. Discrepancy between NTG and POAG with corneal nerves in CCM. *Eye (Lond)*.

Jones, M.A., Marfurt, C.F., 1996. Sympathetic stimulation of corneal epithelial proliferation in wounded and nonwounded rat eyes. *Invest Ophthalmol Vis Sci* 37, 2535-2547.

Joo, M.J., Yuhan, K.R., Hyon, J.Y., Lai, H., Hose, S., Sinha, D., O'Brien, T.P., 2004. The effect of nerve growth factor on corneal sensitivity after laser in situ keratomileusis. *Arch Ophthalmol* 122, 1338-1341.

Jordan, C., Patel, D.V., Abeysekera, N., McGhee, C.N., 2014. In vivo confocal microscopy analyses of corneal microstructural changes in a prospective study of collagen cross-linking in keratoconus. *Ophthalmology* 121, 469-474.

Jung, Y.H., Ryu, J.S., Yoon, C.H., Kim, M.K., 2021. Age-Dependent Distinct Distributions of Dendritic Cells in Autoimmune Dry Eye Murine Model. *Cells* 10.

Kamel, J.T., Zhang, A.C., Downie, L.E., 2019. Corneal Epithelial Dendritic Cell Response as a Putative Marker of Neuro-inflammation in Small Fiber Neuropathy. *Ocul Immunol Inflamm*, 1-4.

Kashem, S.W., Riedl, M.S., Yao, C., Honda, C.N., Vulchanova, L., Kaplan, D.H., 2015. Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. *Immunity* 43, 515-526.

Kawano, H., Kimura-Kuroda, J., Komuta, Y., Yoshioka, N., Li, H.P., Kawamura, K., Li, Y., Raisman, G., 2012. Role of the lesion scar in the response to damage and repair of the central nervous system. *Cell Tissue Res* 349, 169-180.

Kawashima, K., Fujii, T., Moriwaki, Y., Misawa, H., Horiguchi, K., 2015. Non-neuronal cholinergic system in regulation of immune function with a focus on alpha7 nAChRs. *Int Immunopharmacol* 29, 127-134.

Kemp, H.I., Petropoulos, I.N., Rice, A.S.C., Vollert, J., Maier, C., Strum, D., Schargus, M., Peto, T., Hau, S., Chopra, R., Malik, R.A., 2017. Use of Corneal Confocal Microscopy to Evaluate Small Nerve Fibers in Patients With Human Immunodeficiency Virus. *JAMA Ophthalmol* 135, 795-800.

Khalil, M., Babes, A., Lakra, R., Forsch, S., Reeh, P.W., Wirtz, S., Becker, C., Neurath, M.F., Engel, M.A., 2016. Transient receptor potential melastatin 8 ion channel in macrophages modulates colitis through a balance-shift in TNF-alpha and interleukin-10 production. *Mucosal Immunol* 9, 1500-1513.

Kinard, K.I., Smith, A.G., Singleton, J.R., Lessard, M.K., Katz, B.J., Warner, J.E., Crum, A.V., Mifflin, M.D., Brennan, K.C., Digre, K.B., 2015. Chronic migraine is associated with reduced corneal nerve fiber density and symptoms of dry eye. *Headache* 55, 543-549.

Klitsch, A., Evdokimov, D., Frank, J., Thomas, D., Saffer, N., Meyer Zu Altenschildesche, C., Sisignano, M., Kampik, D., Malik, R.A., Sommer, C., Uceyler, N., 2020. Reduced association between dendritic cells and corneal sub-basal nerve fibers in patients with fibromyalgia syndrome. *J Peripher Nerv Syst* 25, 9-18.

Knickelbein, J.E., Buella, K.A., Hendricks, R.L., 2014. Antigen-presenting cells are stratified within normal human corneas and are rapidly mobilized during ex vivo viral infection. *Invest Ophthalmol Vis Sci* 55, 1118-1123.

Knickelbein, J.E., Watkins, S.C., McMenamin, P.G., Hendricks, R.L., 2009. Stratification of Antigen-presenting Cells within the Normal Cornea. *Ophthalmol Eye Dis* 1, 45-54.

Ko, J.A., Yanai, R., Nishida, T., 2009. Up-regulation of ZO-1 expression and barrier function in cultured human corneal epithelial cells by substance P. *FEBS Lett* 583, 2148-2153.

Kovacs, I., Ludany, A., Koszegi, T., Feher, J., Kovacs, B., Szolcsanyi, J., Pinter, E., 2005. Substance P released from sensory nerve endings influences tear secretion and goblet cell function in the rat. *Neuropeptides* 39, 395-402.

Kovacs, I., Luna, C., Quirce, S., Mizerska, K., Callejo, G., Riestra, A., Fernandez-Sanchez, L., Meseguer, V.M., Cuenca, N., Merayo-Llodes, J., Acosta, M.C., Gasull, X., Belmonte, C., Gallar, J., 2016. Abnormal activity of corneal cold thermoreceptors underlies the unpleasant sensations in dry eye disease. *Pain* 157, 399-417.

Kurbanyan, K., Hoesl, L.M., Schrems, W.A., Hamrah, P., 2012. Corneal nerve alterations in acute Acanthamoeba and fungal keratitis: an in vivo confocal microscopy study. *Eye (Lond)* 26, 126-132.

Kwon, M.S., Carnt, N.A., Truong, N.R., Pattamatta, U., White, A.J., Samarawickrama, C., Cunningham, A.L., 2018. Dendritic cells in the cornea during Herpes simplex viral infection and inflammation. *Surv Ophthalmol* 63, 565-578.

Labbe, A., Liang, Q., Wang, Z., Zhang, Y., Xu, L., Baudouin, C., Sun, X., 2013. Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. *Invest Ophthalmol Vis Sci* 54, 5144-5150.

Lagali, N.S., Badian, R.A., Liu, X., Feldreich, T.R., Arnlov, J., Utheim, T.P., Dahlin, L.B., Rolandsson, O., 2018. Dendritic cell maturation in the corneal epithelium with onset of type 2 diabetes is associated with tumor necrosis factor receptor superfamily member 9. *Sci Rep* 8, 14248.

Lambiase, A., Rama, P., Bonini, S., Caprioglio, G., Aloe, L., 1998. Topical treatment with nerve growth factor for corneal neurotrophic ulcers. *N Engl J Med* 338, 1174-1180.

Lasagni Vitar, R.M., Fonteyne, P., Chaabane, L., Rama, P., Ferrari, G., 2021. A Hypothalamic-Controlled Neural Reflex Promotes Corneal Inflammation. *Invest Ophthalmol Vis Sci* 62, 21.

Lasagni Vitar, R.M., Rama, P., Ferrari, G., 2022. The two-faced effects of nerves and neuropeptides in corneal diseases. *Prog Retin Eye Res* 86, 100974.

Launay, P.S., Reboussin, E., Liang, H., Kessal, K., Godefroy, D., Rostene, W., Sahel, J.A., Baudouin, C., Melik Parsadaniantz, S., Reaux Le Goazigo, A., 2016. Ocular inflammation induces trigeminal pain, peripheral and central neuroinflammatory mechanisms. *Neurobiol Dis* 88, 16-28.

Lazarov-Spiegler, O., Solomon, A.S., Zeev-Brann, A.B., Hirschberg, D.L., Lavie, V., Schwartz, M., 1996. Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J* 10, 1296-1302.

Lee, E.J., Rosenbaum, J.T., Planck, S.R., 2010. Epifluorescence intravital microscopy of murine corneal dendritic cells. *Invest Ophthalmol Vis Sci* 51, 2101-2108.

Lee, H.K., Kim, K.W., Ryu, J.S., Jeong, H.J., Lee, S.M., Kim, M.K., 2019. Bilateral Effect of the Unilateral Corneal Nerve Cut on Both Ocular Surface and Lacrimal Gland. *Invest Ophthalmol Vis Sci* 60, 430-441.

Lee, H.S., Hattori, T., Park, E.Y., Stevenson, W., Chauhan, S.K., Dana, R., 2012. Expression of toll-like receptor 4 contributes to corneal inflammation in experimental dry eye disease. *Invest Ophthalmol Vis Sci* 53, 5632-5640.

Lee, S., Shi, X.Q., Fan, A., West, B., Zhang, J., 2018. Targeting macrophage and microglia activation with colony stimulating factor 1 receptor inhibitor is an effective strategy to treat injury-triggered neuropathic pain. *Mol Pain* 14, 1744806918764979.

Lee, S.J., Kim, J.K., Seo, K.Y., Kim, E.K., Lee, H.K., 2006. Comparison of corneal nerve regeneration and sensitivity between LASIK and laser epithelial keratomileusis (LASEK). *Am J Ophthalmol* 141, 1009-1015.

Leppin, K., Behrendt, A.K., Reichard, M., Stachs, O., Guthoff, R.F., Baltrusch, S., Eule, J.C., Vollmar, B., 2014. Diabetes mellitus leads to accumulation of dendritic cells and nerve fiber damage of the subbasal nerve plexus in the cornea. *Invest Ophthalmol Vis Sci* 55, 3603-3615.

Lewis, E.J.H., Perkins, B.A., Lovblom, L.E., Bazinet, R.P., Wolever, T.M.S., Bril, V., 2017. Effect of omega-3 supplementation on neuropathy in type 1 diabetes: A 12-month pilot trial. *Neurology* 88, 2294-2301.

Li, M., Liu, L., Shi, Y., Sun, L., Ma, X., Zou, J., 2021a. Age-related differences in corneal nerve regeneration after SMILE and the mechanism revealed by metabolomics. *Exp Eye Res* 209, 108665.

Li, S., Shi, S., Luo, B., Xia, F., Ha, Y., Merkley, K.H., Motamedi, M., Zhang, W., Liu, H., 2021b. Tauopathy induces degeneration and impairs regeneration of sensory nerves in the cornea. *Exp Eye Res* 215, 108900.

Li, Z., Burns, A.R., Han, L., Rumbaut, R.E., Smith, C.W., 2011a. IL-17 and VEGF are necessary for efficient corneal nerve regeneration. *Am J Pathol* 178, 1106-1116.

Li, Z., Burns, A.R., Miller, S.B., Smith, C.W., 2011b. CCL20, gammadelta T cells, and IL-22 in corneal epithelial healing. *FASEB J* 25, 2659-2668.

Li, Z., Burns, A.R., Rumbaut, R.E., Smith, C.W., 2007. gamma delta T cells are necessary for platelet and neutrophil accumulation in limbal vessels and efficient epithelial repair after corneal abrasion. *Am J Pathol* 171, 838-845.

Li, Z., Burns, A.R., Smith, C.W., 2006. Two waves of neutrophil emigration in response to corneal epithelial abrasion: distinct adhesion molecule requirements. *Invest Ophthalmol Vis Sci* 47, 1947-1955.

Lin, M., Carlson, E., Diaconu, E., Pearlman, E., 2007. CXCL1/KC and CXCL5/LIX are selectively produced by corneal fibroblasts and mediate neutrophil infiltration to the corneal stroma in LPS keratitis. *J Leukoc Biol* 81, 786-792.

Lin, T., Quellier, D., Lamb, J., Voisin, T., Baral, P., Bock, F., Schonberg, A., Mirchev, R., Pier, G., Chiu, I., Gadjeva, M., 2021. *Pseudomonas aeruginosa*-induced nociceptor activation increases susceptibility to infection. *PLoS Pathog* 17, e1009557.

Lin, X., Xu, B., Sun, Y., Zhong, J., Huang, W., Yuan, J., 2014. Comparison of deep anterior lamellar keratoplasty and penetrating keratoplasty with respect to postoperative corneal sensitivity and tear film function. *Graefes Arch Clin Exp Ophthalmol* 252, 1779-1787.

Link, T.M., Park, U., Vonakis, B.M., Raben, D.M., Soloski, M.J., Caterina, M.J., 2010. TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. *Nat Immunol* 11, 232-239.

Liou, J.T., Liu, F.C., Mao, C.C., Lai, Y.S., Day, Y.J., 2011. Inflammation confers dual effects on nociceptive processing in chronic neuropathic pain model. *Anesthesiology* 114, 660-672.

Liu, J., Li, Z., 2021. Resident Innate Immune Cells in the Cornea. *Front Immunol* 12, 620284.

Liu, J., Wu, M., He, J., Xiao, C., Xue, Y., Fu, T., Lin, C., Dong, D., Li, Z., 2018. Antibiotic-Induced Dysbiosis of Gut Microbiota Impairs Corneal Nerve Regeneration by Affecting CCR2-Negative Macrophage Distribution. *Am J Pathol* 188, 2786-2799.

Liu, J., Xue, Y., Dong, D., Xiao, C., Lin, C., Wang, H., Song, F., Fu, T., Wang, Z., Chen, J., Pan, H., Li, Y., Cai, D., Li, Z., 2017. CCR2(-) and CCR2(+) corneal macrophages exhibit distinct characteristics and balance inflammatory responses after epithelial abrasion. *Mucosal Immunol* 10, 1145-1159.

Liu, L., Dana, R., Yin, J., 2020a. Sensory neurons directly promote angiogenesis in response to inflammation via substance P signaling. *FASEB J* 34, 6229-6243.

Liu, Q., Smith, C.W., Zhang, W., Burns, A.R., Li, Z., 2012. NK cells modulate the inflammatory response to corneal epithelial abrasion and thereby support wound healing. *Am J Pathol* 181, 452-462.

Liu, Y.C., Jung, A.S.J., Chin, J.Y., Yang, L.W.Y., Mehta, J.S., 2020b. Cross-sectional Study on Corneal Denervation in Contralateral Eyes Following SMILE Versus LASIK. *J Refract Surg* 36, 653-660.

Lobo, A.M., Agelidis, A.M., Shukla, D., 2019. Pathogenesis of herpes simplex keratitis: The host cell response and ocular surface sequelae to infection and inflammation. *Ocul Surf* 17, 40-49.

Loi, J.K., Alexandre, Y.O., Senthil, K., Schienstock, D., Sandford, S., Devi, S., Christo, S.N., Mackay, L.K., Chinnery, H.R., Osborne, P.B., Downie, L.E., Sloan, E.K., Mueller, S.N., 2022. Corneal tissue-resident memory T cells form a unique immune compartment at the ocular surface. *Cell Rep* 39, 110852.

Luna, C., Quirce, S., Aracil-Marco, A., Belmonte, C., Gallar, J., Acosta, M.C., 2021. Unilateral Corneal Insult Also Alters Sensory Nerve Activity in the Contralateral Eye. *Front Med (Lausanne)* 8, 767967.

Luo, Y., Raible, D., Raper, J.A., 1993. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* 75, 217-227.

Lv, H., Li, A., Zhang, X., Xu, M., Qiao, Y., Zhang, J., Yu, L., 2014. Meta-analysis and review on the changes of tear function and corneal sensitivity in diabetic patients. *Acta Ophthalmol* 92, e96-e104.

Lv, Z., Xu, X., Sun, Z., Yang, Y.X., Guo, H., Li, J., Sun, K., Wu, R., Xu, J., Jiang, Q., Ikegawa, S., Shi, D., 2021. TRPV1 alleviates osteoarthritis by inhibiting M1 macrophage polarization via Ca(2+)/CaMKII/Nrf2 signaling pathway. *Cell Death Dis* 12, 504.

MacIver, M.B., Tanelian, D.L., 1993. Free nerve ending terminal morphology is fiber type specific for A delta and C fibers innervating rabbit corneal epithelium. *J Neurophysiol* 69, 1779-1783.

Malik, R.A., Kallinikos, P., Abbott, C.A., van Schie, C.H., Morgan, P., Efron, N., Boulton, A.J., 2003. Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. *Diabetologia* 46, 683-688.

Mantelli, F., Micera, A., Sacchetti, M., Bonini, S., 2010. Neurogenic inflammation of the ocular surface. *Curr Opin Allergy Clin Immunol* 10, 498-504.

Marco, B., Alessandro, R., Philippe, F., Fabio, B., Paolo, R., Giulio, F., 2018. The Effect of Aging on Nerve Morphology and Substance P Expression in Mouse and Human Corneas. *Invest Ophthalmol Vis Sci* 59, 5329-5335.

Marfurt, C.F., Cox, J., Deek, S., Dvorscak, L., 2010. Anatomy of the human corneal innervation. *Exp Eye Res* 90, 478-492.

Marfurt, C.F., Ellis, L.C., 1993. Immunohistochemical localization of tyrosine hydroxylase in corneal nerves. *J Comp Neurol* 336, 517-531.

Marfurt, C.F., Jones, M.A., Thrasher, K., 1998. Parasympathetic innervation of the rat cornea. *Exp Eye Res* 66, 437-448.

Marfurt, C.F., Kingsley, R.E., Echtenkamp, S.E., 1989. Sensory and sympathetic innervation of the mammalian cornea. A retrograde tracing study. *Invest Ophthalmol Vis Sci* 30, 461-472.

Marquez, A., Guernsey, L.S., Frizzi, K.E., Cundiff, M., Constantino, I., Muttalib, N., Arenas, F., Zhou, X., Lim, S.H., Ferdousi, M., Ponirakis, G., Silverdale, M., Kobylecki, C., Jones, M., Marshall, A., Malik, R.A., Jolival, C.G., 2021. Tau associated peripheral and central neurodegeneration: Identification of an early imaging marker for tauopathy. *Neurobiol Dis* 151, 105273.

Marriott, I., Bost, K.L., 2001. Expression of authentic substance P receptors in murine and human dendritic cells. *J Neuroimmunol* 114, 131-141.

Marrone, M.C., Morabito, A., Giustizieri, M., Chiurchiu, V., Leuti, A., Mattioli, M., Marinelli, S., Riganti, L., Lombardi, M., Murana, E., Totaro, A., Piomelli, D., Ragozzino, D., Oddi, S., Maccarrone, M., Verderio, C., Marinelli, S., 2017. TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. *Nat Commun* 8, 15292.

Marsovszky, L., Resch, M.D., Visontai, Z., Nemeth, J., 2014. Confocal microscopy of epithelial and langerhans cells of the cornea in patients using travoprost drops containing two different preservatives. *Pathol Oncol Res* 20, 741-746.

Martone, G., Frezzotti, P., Tosi, G.M., Traversi, C., Mittica, V., Malandrini, A., Pichierri, P., Balestrazzi, A., Motolese, P.A., Motolese, I., Motolese, E., 2009. An in vivo confocal microscopy analysis of effects of topical antiglaucoma therapy with preservative on corneal innervation and morphology. *Am J Ophthalmol* 147, 725-735 e721.

Maruoka, S., Inaba, M., Ogata, N., 2018. Activation of Dendritic Cells in Dry Eye Mouse Model. *Invest Ophthalmol Vis Sci* 59, 3269-3277.

Mayer, W.J., Irschick, U.M., Moser, P., Wurm, M., Huemer, H.P., Romani, N., Irschick, E.U., 2007. Characterization of antigen-presenting cells in fresh and cultured human corneas using novel dendritic cell markers. *Invest Ophthalmol Vis Sci* 48, 4459-4467.

McClellan, A.J., Volpe, E.A., Zhang, X., Darlington, G.J., Li, D.Q., Pflugfelder, S.C., de Paiva, C.S., 2014. Ocular surface disease and dacryoadenitis in aging C57BL/6 mice. *Am J Pathol* 184, 631-643.

McClellan, S.A., Zhang, Y., Barrett, R.P., Hazlett, L.D., 2008. Substance P promotes susceptibility to *Pseudomonas aeruginosa* keratitis in resistant mice: anti-inflammatory mediators downregulated. *Invest Ophthalmol Vis Sci* 49, 1502-1511.

McDougal, D.H., Gamlin, P.D., 2015. Autonomic control of the eye. *Compr Physiol* 5, 439-473.

McMahon, S., Koltzenburg, M., 1990. The changing role of primary afferent neurones in pain. *Pain* 43, 269-272.

Medeiros, C.S., Marino, G.K., Lassance, L., Thangavadivel, S., Santhiago, M.R., Wilson, S.E., 2018. The Impact of Photorefractive Keratectomy and Mitomycin C on Corneal Nerves and Their Regeneration. *J Refract Surg* 34, 790-798.

Messmer, E.M., Schmid-Tannwald, C., Zapp, D., Kampik, A., 2010. In vivo confocal microscopy of corneal small fiber damage in diabetes mellitus. *Graefes Arch Clin Exp Ophthalmol* 248, 1307-1312.

Mikulec, A.A., Tanelian, D.L., 1996. CGRP increases the rate of corneal re-epithelialization in an in vitro whole mount preparation. *J Ocul Pharmacol Ther* 12, 417-423.

Misra, S.L., Goh, Y.W., Patel, D.V., Riley, A.F., McGhee, C.N., 2015. Corneal microstructural changes in nerve fiber, endothelial and epithelial density after cataract surgery in patients with diabetes mellitus. *Cornea* 34, 177-181.

Misra, S.L., Kersten, H.M., Roxburgh, R.H., Danesh-Meyer, H.V., McGhee, C.N., 2017. Corneal nerve microstructure in Parkinson's disease. *J Clin Neurosci* 39, 53-58.

Miyake, T., Shirakawa, H., Nakagawa, T., Kaneko, S., 2015. Activation of mitochondrial transient receptor potential vanilloid 1 channel contributes to microglial migration. *Glia* 63, 1870-1882.

Moalem, G., Tracey, D.J., 2006. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res Rev* 51, 240-264.

Moein, H.R., Kheirkhah, A., Muller, R.T., Cruzat, A.C., Pavan-Langston, D., Hamrah, P., 2018. Corneal nerve regeneration after herpes simplex keratitis: A longitudinal in vivo confocal microscopy study. *Ocul Surf* 16, 218-225.

Moilanen, J.A., Holopainen, J.M., Vesaluoma, M.H., Tervo, T.M., 2008. Corneal recovery after lasik for high myopia: a 2-year prospective confocal microscopic study. *Br J Ophthalmol* 92, 1397-1402.

Morelli, A.E., Sumpter, T.L., Rojas-Canales, D.M., Bandyopadhyay, M., Chen, Z., Tkacheva, O., Shufesky, W.J., Wallace, C.T., Watkins, S.C., Berger, A., Paige, C.J., Faló, L.D., Jr., Larregina, A.T., 2020. Neurokinin-1 Receptor Signaling Is Required for Efficient Ca(2+) Flux in T-Cell-Receptor-Activated T Cells. *Cell Rep* 30, 3448-3465 e3448.

Morgan, C., DeGroat, W.C., Jannetta, P.J., 1987. Sympathetic innervation of the cornea from the superior cervical ganglion. An HRP study in the cat. *J Auton Nerv Syst* 20, 179-183.

Motterle, L., Diebold, Y., Enriquez de Salamanca, A., Saez, V., Garcia-Vazquez, C., Stern, M.E., Calonge, M., Leonardi, A., 2006. Altered expression of neurotransmitter receptors and neuromediators in vernal keratoconjunctivitis. *Arch Ophthalmol* 124, 462-468.

Muller, L.J., Marfurt, C.F., Kruse, F., Tervo, T.M., 2003. Corneal nerves: structure, contents and function. *Exp Eye Res* 76, 521-542.

Muller, L.J., Pels, L., Vrensen, G.F., 1996. Ultrastructural organization of human corneal nerves. *Invest Ophthalmol Vis Sci* 37, 476-488.

Muller, R.T., Abedi, F., Cruzat, A., Witkin, D., Baniyadi, N., Cavalcanti, B.M., Jamali, A., Chodosh, J., Dana, R., Pavan-Langston, D., Hamrah, P., 2015. Degeneration and Regeneration of Subbasal Corneal Nerves after Infectious Keratitis: A Longitudinal In Vivo Confocal Microscopy Study. *Ophthalmology* 122, 2200-2209.

Murata, Y., Masuko, S., 2006. Peripheral and central distribution of TRPV1, substance P and CGRP of rat corneal neurons. *Brain Res* 1085, 87-94.

Nagano, T., Nakamura, M., Nakata, K., Yamaguchi, T., Takase, K., Okahara, A., Ikuse, T., Nishida, T., 2003. Effects of substance P and IGF-1 in corneal epithelial barrier function and wound healing in a rat model of neurotrophic keratopathy. *Invest Ophthalmol Vis Sci* 44, 3810-3815.

Namavari, A., Chaudhary, S., Ozturk, O., Chang, J.H., Yco, L., Sonawane, S., Katam, N., Khanolkar, V., Hallak, J., Sarkar, J., Jain, S., 2012. Semaphorin 7a links nerve regeneration and inflammation in the cornea. *Invest Ophthalmol Vis Sci* 53, 4575-4585.

Namikawa, K., Okamoto, T., Suzuki, A., Konishi, H., Kiyama, H., 2006. Pancreatitis-associated protein-III is a novel macrophage chemoattractant implicated in nerve regeneration. *J Neurosci* 26, 7460-7467.

NCT01756456, Evaluation of Safety and Efficacy of rhNGF in Patients With Stage 2 and 3 Neurotrophic Keratitis. <https://ClinicalTrials.gov/show/NCT01756456>.

NCT02227147, Evaluation of Efficacy of 20 µg/ml rhNGF New Formulation (With Anti-oxidant) in Patients With Stage 2 and 3 NK. <https://ClinicalTrials.gov/show/NCT02227147>.

NCT03084861, A Clinical Trial to Asses Efficacy and Safety of Cord Blood Eye Drops in Neurotrophic Keratopathy. <https://ClinicalTrials.gov/show/NCT03084861>.

Neelam, S., Mellon, J., Wilkerson, A., Niederkorn, J.Y., 2018. Induction of Contrasuppressor Cells and Loss of Immune Privilege Produced by Corneal Nerve Ablation. *Invest Ophthalmol Vis Sci* 59, 4738-4747.

Ng, S.M., Ren, M., Lindsley, K.B., Hawkins, B.S., Kuo, I.C., 2021. Transepithelial versus epithelium-off corneal crosslinking for progressive keratoconus. *Cochrane Database Syst Rev* 3, CD013512.

Niederer, R.L., McGhee, C.N., 2010. Clinical in vivo confocal microscopy of the human cornea in health and disease. *Prog Retin Eye Res* 29, 30-58.

Niederer, R.L., Perumal, D., Sherwin, T., McGhee, C.N., 2007. Corneal innervation and cellular changes after corneal transplantation: an in vivo confocal microscopy study. *Invest Ophthalmol Vis Sci* 48, 621-626.

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., Littman, D.R., Reinecker, H.C., 2005. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307, 254-258.

Nishida, T., 2005. Neurotrophic mediators and corneal wound healing. *Ocul Surf* 3, 194-202.

Ochando, J.C., Homma, C., Yang, Y., Hidalgo, A., Garin, A., Tacke, F., Angeli, V., Li, Y., Boros, P., Ding, Y., Jessberger, R., Trinchieri, G., Lira, S.A., Randolph, G.J., Bromberg, J.S., 2006. Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. *Nat Immunol* 7, 652-662.

Oh, J.Y., Choi, H., Lee, R.H., Roddy, G.W., Ylostalo, J.H., Wawrousek, E., Prockop, D.J., 2012. Identification of the HSPB4/TLR2/NF-kappaB axis in macrophage as a therapeutic target for sterile inflammation of the cornea. *EMBO molecular medicine* 4, 435-448.

Okada, Y., Sumioka, T., Ichikawa, K., Sano, H., Nambu, A., Kobayashi, K., Uchida, K., Suzuki, Y., Tominaga, M., Reinach, P.S., Hirai, S.I., Jester, J.V., Miyajima, M., Shirai, K., Iwanishi, H., Kao, W.W., Liu, C.Y., Saika, S., 2019. Sensory nerve supports epithelial stem cell function in healing of corneal epithelium in mice: the role of trigeminal nerve transient receptor potential vanilloid 4. *Lab Invest* 99, 210-230.

Omoto, M., Yoshida, S., Miyashita, H., Kawakita, T., Yoshida, K., Kishino, A., Kimura, T., Shibata, S., Tsubota, K., Okano, H., Shimmura, S., 2012. The semaphorin 3A inhibitor SM-345431 accelerates peripheral nerve regeneration and sensitivity in a murine corneal transplantation model. *PLoS One* 7, e47716.

Pal-Ghosh, S., Blanco, T., Tadvalkar, G., Pajooresh-Ganji, A., Parthasarathy, A., Zieske, J.D., Stepp, M.A., 2011. MMP9 cleavage of the beta4 integrin ectodomain leads to recurrent epithelial erosions in mice. *J Cell Sci* 124, 2666-2675.

Parlanti, P., Pal-Ghosh, S., Williams, A., Tadvalkar, G., Popratiloff, A., Stepp, M.A., 2020. Axonal debris accumulates in corneal epithelial cells after intraepithelial corneal nerves are damaged: A focused Ion Beam Scanning Electron Microscopy (FIB-SEM) study. *Exp Eye Res* 194, 107998.

Parra, A., Madrid, R., Echevarria, D., del Olmo, S., Morenilla-Palao, C., Acosta, M.C., Gallar, J., Dhaka, A., Viana, F., Belmonte, C., 2010. Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. *Nat Med* 16, 1396-1399.

Pasterkamp, R.J., 2012. Getting neural circuits into shape with semaphorins. *Nat Rev Neurosci* 13, 605-618.

Patapoutian, A., Tate, S., Woolf, C.J., 2009. Transient receptor potential channels: targeting pain at the source. *Nat Rev Drug Discov* 8, 55-68.

Patel, D.V., McGhee, C.N., 2013. Quantitative analysis of in vivo confocal microscopy images: a review. *Surv Ophthalmol* 58, 466-475.

Persson, A.K., Liu, S., Faber, C.G., Merkies, I.S., Black, J.A., Waxman, S.G., 2013. Neuropathy-associated Nav1.7 variant I228M impairs integrity of dorsal root ganglion neuron axons. *Ann Neurol* 73, 140-145.

Petropoulos, I.N., Ponirakis, G., Khan, A., Gad, H., Almuhammad, H., Brines, M., Cerami, A., Malik, R.A., 2020. Corneal confocal microscopy: ready for prime time. *Clin Exp Optom* 103, 265-277.

Pflugfelder, S.C., Massaro-Giordano, M., Perez, V.L., Hamrah, P., Deng, S.X., Espandar, L., Foster, C.S., Affeldt, J., Seedor, J.A., Afshari, N.A., Chao, W., Allegretti, M., Mantelli, F., Dana, R., 2020. Topical Recombinant Human Nerve Growth Factor (Cenergermin) for Neurotrophic Keratopathy: A Multicenter Randomized Vehicle-Controlled Pivotal Trial. *Ophthalmology* 127, 14-26.

Ponirakis, G., Al Hamad, H., Sankaranarayanan, A., Khan, A., Chandran, M., Ramadan, M., Tosino, R., Gawhale, P.V., Alobaidi, M., AlSulaiti, E., Elsotouhy, A., Elorabi, M., Khan, S., Nadukkandiyil, N., Osman, S., Thodi, N., Almuhammad, H., Gad, H., Mahfoud, Z.R., Al-Shibani, F., Petropoulos, I.N., Own,



A., Al Kuwari, M., Shuaib, A., Malik, R.A., 2019. Association of corneal nerve fiber measures with cognitive function in dementia. *Ann Clin Transl Neurol* 6, 689-697.

Qi, H., Chuang, E.Y., Yoon, K.C., de Paiva, C.S., Shine, H.D., Jones, D.B., Pflugfelder, S.C., Li, D.Q., 2007. Patterned expression of neurotrophic factors and receptors in human limbal and corneal regions. *Mol Vis* 13, 1934-1941.

Qureshi, S., Ferguson, T.J., Lim, M., You, J.Y., Goshe, J.M., Hood, C.T., 2022. Acute Calcific Band Keratopathy as an Adverse Effect of Recombinant Human Nerve Growth Factor (Cenergermin): A Multicenter Case Series. *Cornea* 41, 52-59.

Ramirez, M., Martinez-Martinez, L.A., Hernandez-Quintela, E., Velazco-Casapia, J., Vargas, A., Martinez-Lavin, M., 2015. Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy. *Semin Arthritis Rheum* 45, 214-219.

Ranno, S., Fogagnolo, P., Rossetti, L., Orzalesi, N., Nucci, P., 2011. Changes in corneal parameters at confocal microscopy in treated glaucoma patients. *Clin Ophthalmol* 5, 1037-1042.

Rao, G.N., John, T., Ishida, N., Aquavella, J.V., 1985. Recovery of corneal sensitivity in grafts following penetrating keratoplasty. *Ophthalmology* 92, 1408-1411.

Reizis, B., Bunin, A., Ghosh, H.S., Lewis, K.L., Sisirak, V., 2011. Plasmacytoid dendritic cells: recent progress and open questions. *Annu Rev Immunol* 29, 163-183.

Richter, A., Slowik, C., Somodi, S., Vick, H.P., Guthoff, R., 1996. Corneal reinnervation following penetrating keratoplasty--correlation of esthesiometry and confocal microscopy. *Ger J Ophthalmol* 5, 513-517.

Robson, L.G., Dyll, S., Sidloff, D., Michael-Titus, A.T., 2010. Omega-3 polyunsaturated fatty acids increase the neurite outgrowth of rat sensory neurones throughout development and in aged animals. *Neurobiol Aging* 31, 678-687.

Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdes-Ferrer, S.I., Levine, Y.A., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., Mak, T.W., Tracey, K.J., 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 334, 98-101.

Rosenberg, M.E., Tervo, T.M., Immonen, I.J., Muller, L.J., Gronhagen-Riska, C., Vesaluoma, M.H., 2000. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 41, 2915-2921.

Roszkowska, A.M., Wylegala, A., Gargano, R., Spinella, R., Inferrera, L., Orzechowska-Wylegala, B., Aragona, P., 2021. Impact of corneal parameters, refractive error and age on density and morphology of the subbasal nerve plexus fibers in healthy adults. *Sci Rep* 11, 6076.

Sacchetti, M., Lambiase, A., 2017. Neurotrophic factors and corneal nerve regeneration. *Neural Regen Res* 12, 1220-1224.

Sadighi Akha, A.A., 2018. Aging and the immune system: An overview. *J Immunol Methods* 463, 21-26.

Sarkar, J., Chaudhary, S., Jassim, S.H., Ozturk, O., Chamon, W., Ganesh, B., Tibrewal, S., Gandhi, S., Byun, Y.S., Hallak, J., Mahmud, D.L., Mahmud, N., Rondelli, D., Jain, S., 2013. CD11b+GR1+ myeloid cells secrete NGF and promote trigeminal ganglion neurite growth: implications for corneal nerve regeneration. *Invest Ophthalmol Vis Sci* 54, 5920-5936.

Sarkar, J., Chaudhary, S., Namavari, A., Ozturk, O., Chang, J.H., Yco, L., Sonawane, S., Khanolkar, V., Hallak, J., Jain, S., 2012. Corneal neurotoxicity due to topical benzalkonium chloride. *Invest Ophthalmol Vis Sci* 53, 1792-1802.

Scapini, P., Lapinet-Vera, J.A., Gasperini, S., Calzetti, F., Bazzoni, F., Cassatella, M.A., 2000. The neutrophil as a cellular source of chemokines. *Immunol Rev* 177, 195-203.

Schain, A.J., Melo-Carrillo, A., Borsook, D., Grutzendler, J., Strassman, A.M., Burstein, R., 2018. Activation of pial and dural macrophages and dendritic cells by cortical spreading depression. *Ann Neurol* 83, 508-521.

Schaumburg, C.S., Siemasko, K.F., De Paiva, C.S., Wheeler, L.A., Niederkorn, J.Y., Pflugfelder, S.C., Stern, M.E., 2011. Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. *J Immunol* 187, 3653-3662.

Scheiblich, H., Trombly, M., Ramirez, A., Heneka, M.T., 2020. Neuroimmune Connections in Aging and Neurodegenerative Diseases. *Trends Immunol* 41, 300-312.

Sekundo, W., Kunert, K.S., Blum, M., 2011. Small incision corneal refractive surgery using the small incision lenticule extraction (SMILE) procedure for the correction of myopia and myopic astigmatism: results of a 6 month prospective study. *Br J Ophthalmol* 95, 335-339.

Semeraro, F., Forbice, E., Nascimbeni, G., Taglietti, M., Romano, V., Guerra, G., Costagliola, C., 2016. Effect of Autologous Serum Eye Drops in Patients with Sjogren Syndrome-related Dry Eye: Clinical and In Vivo Confocal Microscopy Evaluation of the Ocular Surface. *In Vivo* 30, 931-938.

Semeraro, F., Forbice, E., Romano, V., Angi, M., Romano, M.R., Filippelli, M.E., Di Iorio, R., Costagliola, C., 2014. Neurotrophic keratitis. *Ophthalmologica* 231, 191-197.

Senthil, K., Jiao, H., Downie, L.E., Chinnery, H.R., 2021. Altered Corneal Epithelial Dendritic Cell Morphology and Phenotype Following Acute Exposure to Hyperosmolar Saline. *Invest Ophthalmol Vis Sci* 62, 38.

Severo Bem Junior, L., do Rego Aquino, P.L., Nunes Rabelo, N., do Rego Aquino, M.A., Veiga Silva, A.C., Ferreira Valenca Mota, R.C., Rocha Cirne de Azevedo Filho, H., 2020. SARS-CoV-2 and Nervous System - Neurological Manifestations in Patients With COVID-19: A Systematic Review. *J Neurol Res* 10, 113-121.

Seyed-Razavi, Y., Chinnery, H.R., McMenamin, P.G., 2014. A novel association between resident tissue macrophages and nerves in the peripheral stroma of the murine cornea. *Invest Ophthalmol Vis Sci* 55, 1313-1320.

Seyed-Razavi, Y., Lopez, M.J., Mantopoulos, D., Zheng, L., Massberg, S., Sendra, V.G., Harris, D.L., Hamrah, P., 2019. Kinetics of corneal leukocytes by intravitral multiphoton microscopy. *FASEB J* 33, 2199-2211.

Shaheen, B.S., Bakir, M., Jain, S., 2014. Corneal nerves in health and disease. *Surv Ophthalmol* 59, 263-285.

Shetty, R., Sethu, S., Deshmukh, R., Deshpande, K., Ghosh, A., Agrawal, A., Shroff, R., 2016. Corneal Dendritic Cell Density Is Associated with Subbasal Nerve Plexus Features, Ocular Surface Disease Index, and Serum Vitamin D in Evaporative Dry Eye Disease. *Biomed Res Int* 2016, 4369750.

Simsek, C., Kojima, T., Dogru, M., Tsubota, K., 2018. Alterations of Murine Subbasal Corneal Nerves After Environmental Dry Eye Stress. *Invest Ophthalmol Vis Sci* 59, 1986-1995.

Skelsey, M.E., Mellon, J., Niederkorn, J.Y., 2001. Gamma delta T cells are needed for ocular immune privilege and corneal graft survival. *J Immunol* 166, 4327-4333.

Sloniecka, M., Le Roux, S., Boman, P., Bystrom, B., Zhou, Q., Danielson, P., 2015. Expression Profiles of Neuropeptides, Neurotransmitters, and Their Receptors in Human Keratocytes In Vitro and In Situ. *PLoS One* 10, e0134157.

Sosnova, M., Bradl, M., Forrester, J.V., 2005. CD34+ corneal stromal cells are bone marrow-derived and express hemopoietic stem cell markers. *Stem Cells* 23, 507-515.

Spadea, L., Salvatore, S., Paroli, M.P., Vingolo, E.M., 2015. Recovery of corneal sensitivity after collagen crosslinking with and without epithelial debridement in eyes with keratoconus. *J Cataract Refract Surg* 41, 527-532.

St Leger, A.J., Hendricks, R.L., 2011. CD8+ T cells patrol HSV-1-infected trigeminal ganglia and prevent viral reactivation. *J Neurovirol* 17, 528-534.

Staaf, S., Oerther, S., Lucas, G., Mattsson, J.P., Ernfors, P., 2009. Differential regulation of TRP channels in a rat model of neuropathic pain. *Pain* 144, 187-199.

Stepp, M.A., Pal-Ghosh, S., Downie, L.E., Zhang, A.C., Chinnery, H.R., Machet, J., Di Girolamo, N., 2020. Corneal Epithelial "Neuromas": A Case of Mistaken Identity? *Cornea* 39, 930-934.

Stepp, M.A., Pal-Ghosh, S., Tadvalkar, G., Williams, A., Pflugfelder, S.C., de Paiva, C.S., 2018. Reduced intraepithelial corneal nerve density and sensitivity accompany desiccating stress and aging in C57BL/6 mice. *Exp Eye Res* 169, 91-98.

Stepp, M.A., Tadvalkar, G., Hakh, R., Pal-Ghosh, S., 2017. Corneal epithelial cells function as surrogate Schwann cells for their sensory nerves. *Glia* 65, 851-863.

Stern, M.E., Schaumburg, C.S., Pflugfelder, S.C., 2013. Dry eye as a mucosal autoimmune disease. *Int Rev Immunol* 32, 19-41.

Stettner, M., Hinrichs, L., Guthoff, R., Bairov, S., Petropoulos, I.N., Warnke, C., Hartung, H.P., Malik, R.A., Kieseier, B.C., 2016. Corneal confocal microscopy in chronic inflammatory demyelinating polyneuropathy. *Ann Clin Transl Neurol* 3, 88-100.

Streilein, J.W., Toews, G.B., Bergstresser, P.R., 1979. Corneal allografts fail to express Ia antigens. *Nature* 282, 326-327.

Sui, T., Zhang, J., Du, S., Su, C., Que, J., Cao, X., 2014. Potential risk of mitomycin C at high concentrations on peripheral nerve structure. *Neural Regen Res* 9, 821-827.

Sun, T., Nguyen, A., Gommerman, J.L., 2020. Dendritic Cell Subsets in Intestinal Immunity and Inflammation. *J Immunol* 204, 1075-1083.

Suvas, S., 2017. Role of Substance P Neuropeptide in Inflammation, Wound Healing, and Tissue Homeostasis. *J Immunol* 199, 1543-1552.

Takekani, Y., Marmalidou, A., Dohlman, T.H., Singh, R.B., Amouzegar, A., Chauhan, S.K., Chen, Y., Dana, R., 2020. Restoration of Regulatory T-Cell Function in Dry Eye Disease by Antagonizing Substance P/Neurokinin-1 Receptor. *Am J Pathol* 190, 1859-1866.

Tavakoli, M., Boulton, A.J., Efron, N., Malik, R.A., 2011. Increased Langerhan cell density and corneal nerve damage in diabetic patients: role of immune mechanisms in human diabetic neuropathy. *Contact lens & anterior eye : the journal of the British Contact Lens Association* 34, 7-11.

Taylor, A.W., 2007. Ocular immunosuppressive microenvironment. *Chem Immunol Allergy* 92, 71-85.

Tepelus, T.C., Chiu, G.B., Huang, J., Huang, P., Sadda, S.R., Irvine, J., Lee, O.L., 2017a. Correlation between corneal innervation and inflammation evaluated with confocal microscopy and symptomatology in patients with dry eye syndromes: a preliminary study. *Graefes Arch Clin Exp Ophthalmol* 255, 1771-1778.

Tepelus, T.C., Huang, J., Sadda, S.R., Lee, O.L., 2017b. Characterization of Corneal Involvement in Eyes With Mucous Membrane Pemphigoid by In Vivo Confocal Microscopy. *Cornea* 36, 933-941.

Tervo, T., Palkama, A., 1978. Innervation of the rabbit cornea. A histochemical and electron-microscopic study. *Acta Anat (Basel)* 102, 164-175.

Tian, X., Wang, T., Zhang, S., Wang, Q., Hu, X., Ge, C., Xie, L., Zhou, Q., 2018. PEDF Reduces the Severity of Herpetic Simplex Keratitis in Mice. *Invest Ophthalmol Vis Sci* 59, 2923-2931.

Toivanen, M., Tervo, T., Partanen, M., Vannas, A., Hervonen, A., 1987. Histochemical demonstration of adrenergic nerves in the stroma of human cornea. *Invest Ophthalmol Vis Sci* 28, 398-400.

Tummanapalli, S.S., Willcox, M.D.P., Issar, T., Kwai, N., Poynten, A.M., Krishnan, A.V., Pisarcikova, J., Markoulli, M., 2020. The Effect of Age, Gender and Body Mass Index on Tear Film Neuromediators and Corneal Nerves. *Curr Eye Res* 45, 411-418.

Ueno, H., Ferrari, G., Hattori, T., Saban, D.R., Katikireddy, K.R., Chauhan, S.K., Dana, R., 2012. Dependence of corneal stem/progenitor cells on ocular surface innervation. *Invest Ophthalmol Vis Sci* 53, 867-872.

Valladeau, J., Ravel, O., Dezutter-Dambuyant, C., Moore, K., Kleijmeer, M., Liu, Y., Duvert-Frances, V., Vincent, C., Schmitt, D., Davoust, J., Caux, C., Lebecque, S., Saeland, S., 2000. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12, 71-81.

Veres, T.Z., Shevchenko, M., Krasteva, G., Spies, E., Prenzler, F., Rochlitzer, S., Tschernig, T., Krug, N., Kummer, W., Braun, A., 2009. Dendritic cell-nerve clusters are sites of T cell proliferation in allergic airway inflammation. *Am J Pathol* 174, 808-817.

Villani, E., Galimberti, D., Viola, F., Mapelli, C., Del Papa, N., Ratiglia, R., 2008. Corneal involvement in rheumatoid arthritis: an in vivo confocal study. *Invest Ophthalmol Vis Sci* 49, 560-564.

Villani, E., Magnani, F., Viola, F., Santaniello, A., Scorza, R., Nucci, P., Ratiglia, R., 2013. In vivo confocal evaluation of the ocular surface morpho-functional unit in dry eye. *Optom Vis Sci* 90, 576-586.

Villani, E., Sacchi, M., Magnani, F., Nicodemo, A., Williams, S.E., Rossi, A., Ratiglia, R., De Cilla, S., Nucci, P., 2016. The Ocular Surface in Medically Controlled Glaucoma: An In Vivo Confocal Study. *Invest Ophthalmol Vis Sci* 57, 1003-1010.

Villani, E., Viola, F., Sala, R., Salvi, M., Mapelli, C., Curro, N., Vannucchi, G., Beck-Peccoz, P., Ratiglia, R., 2010. Corneal involvement in Graves' orbitopathy: an in vivo confocal study. *Invest Ophthalmol Vis Sci* 51, 4574-4578.

Vukovic, J., Blomster, L.V., Chinnery, H.R., Weninger, W., Jung, S., McMenemy, P.G., Ruitenberg, M.J., 2010. Bone marrow chimeric mice reveal a role for CX(3)CR1 in maintenance of the monocyte-derived cell population in the olfactory neuroepithelium. *J Leukoc Biol* 88, 645-654.

Wan, D., Du, T., Hong, W., Chen, L., Que, H., Lu, S., Peng, X., 2021a. Neurological complications and infection mechanism of SARS-COV-2. *Signal Transduct Target Ther* 6, 406.

Wan, L., Bai, X., Zhou, Q., Chen, C., Wang, H., Liu, T., Xue, J., Wei, C., Xie, L., 2022. The advanced glycation end-products (AGEs)/ROS/NLRP3 inflammasome axis contributes to delayed diabetic corneal wound healing and nerve regeneration. *Int J Biol Sci* 18, 809-825.

Wan, S.J., Datta, A., Flandrin, O., Metruccio, M.M.E., Ma, S., Nieto, V., Kroken, A.R., Hill, R.Z., Bautista, D.M., Evans, D.J., Fleiszig, S.M.J., 2021b. Nerve-associated transient receptor potential ion channels can contribute to intrinsic resistance to bacterial adhesion in vivo. *FASEB J* 35, e21899.

Wang, E.F., Misra, S.L., Patel, D.V., 2015. In Vivo Confocal Microscopy of the Human Cornea in the Assessment of Peripheral Neuropathy and Systemic Diseases. *Biomed Res Int* 2015, 951081.

Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C.J., Tracey, K.J., 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421, 384-388.

Wang, X., Li, W., Zhou, Q., Li, J., Wang, X., Zhang, J., Li, D., Qi, X., Liu, T., Zhao, X., Li, S., Yang, L., Xie, L., 2020. MANF Promotes Diabetic Corneal Epithelial Wound Healing and Nerve Regeneration by Attenuating Hyperglycemia-Induced Endoplasmic Reticulum Stress. *Diabetes* 69, 1264-1278.

Wang, Y., Wan, L., Zhang, Z., Li, J., Qu, M., Zhou, Q., 2021. Topical calcitriol application promotes diabetic corneal wound healing and reinnervation through inhibiting NLRP3 inflammasome activation. *Exp Eye Res* 209, 108668.

Wang, Y., Zhao, F., Zhu, W., Xu, J., Zheng, T., Sun, X., 2010. In vivo confocal microscopic evaluation of morphologic changes and dendritic cell distribution in pterygium. *Am J Ophthalmol* 150, 650-655 e651.

Wei, S., Qiu, C.Y., Jin, Y., Liu, T.T., Hu, W.P., 2021. TNF-alpha acutely enhances acid-sensing ion channel currents in rat dorsal root ganglion neurons via a p38 MAPK pathway. *J Neuroinflammation* 18, 92.

Wieghofer, P., Hagemeyer, N., Sankowski, R., Schlecht, A., Staszewski, O., Amann, L., Gruber, M., Koch, J., Hausmann, A., Zhang, P., Boneva, S., Masuda, T., Hilgendorf, I., Goldmann, T., Bottcher, C., Priller, J., Rossi, F.M., Lange, C., Prinz, M., 2021. Mapping the origin and fate of myeloid cells in distinct compartments of the eye by single-cell profiling. *EMBO J* 40, e105123.

Wilson, S.E., Ambrosio, R., 2001. Laser in situ keratomileusis-induced neurotrophic epitheliopathy. *Am J Ophthalmol* 132, 405-406.

Wu, M., Downie, L.E., Grover, L.M., Moakes, R.J.A., Rauz, S., Logan, A., Jiao, H., Hill, L.J., Chinnery, H.R., 2020. The neuroregenerative effects of topical decorin on the injured mouse cornea. *Journal of neuroinflammation* 17, 142.

Xu, J., Chen, P., Yu, C., Liu, Y., Hu, S., Di, G., 2021. In vivo Confocal Microscopic Evaluation of Corneal Dendritic Cell Density and Subbasal Nerve Parameters in Dry Eye Patients: A Systematic Review and Meta-analysis. *Front Med (Lausanne)* 8, 578233.

Xu, Y., Kapp, J.A., 2001. gammadelta T cells are critical for the induction of anterior chamber-associated immune deviation. *Immunology* 104, 142-148.

Xue, Y., He, J., Xiao, C., Guo, Y., Fu, T., Liu, J., Lin, C., Wu, M., Yang, Y., Dong, D., Pan, H., Xia, C., Ren, L., Li, Z., 2018. The mouse autonomic nervous system modulates inflammation and epithelial

renewal after corneal abrasion through the activation of distinct local macrophages. *Mucosal Immunol* 11, 1496-1511.

Yamada, N., Matsuda, R., Morishige, N., Yanai, R., Chikama, T.I., Nishida, T., Ishimitsu, T., Kamiya, A., 2008. Open clinical study of eye-drops containing tetrapeptides derived from substance P and insulin-like growth factor-1 for treatment of persistent corneal epithelial defects associated with neurotrophic keratopathy. *Br J Ophthalmol* 92, 896-900.

Yamagami, S., Yokoo, S., Usui, T., Yamagami, H., Amano, S., Ebihara, N., 2005. Distinct populations of dendritic cells in the normal human donor corneal epithelium. *Invest Ophthalmol Vis Sci* 46, 4489-4494.

Yamaguchi, T., Calvacanti, B.M., Cruzat, A., Qazi, Y., Ishikawa, S., Osuka, A., Lederer, J., Hamrah, P., 2014. Correlation between human tear cytokine levels and cellular corneal changes in patients with bacterial keratitis by in vivo confocal microscopy. *Invest Ophthalmol Vis Sci* 55, 7457-7466.

Yamakawa, M., Santosa, S.M., Chawla, N., Ivakhnitskaia, E., Del Pino, M., Giakas, S., Nadel, A., Bontu, S., Tambe, A., Guo, K., Han, K.Y., Cortina, M.S., Yu, C., Rosenblatt, M.I., Chang, J.H., Azar, D.T., 2020. Transgenic models for investigating the nervous system: Currently available neurofluorescent reporters and potential neuronal markers. *Biochim Biophys Acta Gen Subj* 1864, 129595.

Yamazaki, R., Yamazoe, K., Yoshida, S., Hatou, S., Inagaki, E., Okano, H., Tsubota, K., Shimmura, S., 2017. The Semaphorin 3A inhibitor SM-345431 preserves corneal nerve and epithelial integrity in a murine dry eye model. *Sci Rep* 7, 15584.

Yanai, R., Nishida, T., Hatano, M., Uchi, S.H., Yamada, N., Kimura, K., 2020. Role of the Neurokinin-1 Receptor in the Promotion of Corneal Epithelial Wound Healing by the Peptides FGLM-NH2 and SSSR in Neurotrophic Keratopathy. *Invest Ophthalmol Vis Sci* 61, 29.

Yang, X., Gong, H., Liu, Z., Liu, H., Wang, H., Li, Z., 2010. Similar and different effects of capsaicin and resiniferatoxin on substance P release and transient receptor potential vanilloid type 1 expression of cultured rat dorsal root ganglion neurons. *Methods Find Exp Clin Pharmacol* 32, 3-11.

Yin, M., Li, C., Peng, X.D., Zhao, G.Q., Wu, Y., Zheng, H.R., Wang, Q., Xu, Q., Jiang, N., 2019. Expression and role of calcitonin gene-related peptide in mouse *Aspergillus fumigatus* keratitis. *Int J Ophthalmol* 12, 697-704.

Yoo, B.B., Mazmanian, S.K., 2017. The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut. *Immunity* 46, 910-926.

Yoon, H.J., Jang, W.H., An, S., Ji, Y.S., Yoon, K.C., 2022. Tear Neuromediators in Subjects with and without Dry Eye According to Ocular Sensitivity. *Chonnam Med J* 58, 37-42.

You, L., Kruse, F.E., Volcker, H.E., 2000. Neurotrophic factors in the human cornea. *Invest Ophthalmol Vis Sci* 41, 692-702.

Yu, C.Q., Rosenblatt, M.I., 2007. Transgenic corneal neurofluorescence in mice: a new model for in vivo investigation of nerve structure and regeneration. *Invest Ophthalmol Vis Sci* 48, 1535-1542.

Yu, F.X., Lee, P.S.Y., Yang, L., Gao, N., Zhang, Y., Ljubimov, A.V., Yang, E., Zhou, Q., Xie, L., 2022. The impact of sensory neuropathy and inflammation on epithelial wound healing in diabetic corneas. *Prog Retin Eye Res*, 101039.

Yu, M., Lee, S.M., Lee, H., Amouzegar, A., Nakao, T., Chen, Y., Dana, R., 2020. Neurokinin-1 Receptor Antagonism Ameliorates Dry Eye Disease by Inhibiting Antigen-Presenting Cell Maturation and T Helper 17 Cell Activation. *Am J Pathol* 190, 125-133.

Yuan, K., Zheng, J., Shen, X., Wu, Y., Han, Y., Jin, X., Huang, X., 2022. Sensory nerves promote corneal inflammation resolution via CGRP mediated transformation of macrophages to the M2 phenotype through the PI3K/AKT signaling pathway. *Int Immunopharmacol* 102, 108426.

Yun, H., Lathrop, K.L., Hendricks, R.L., 2016. A Central Role for Sympathetic Nerves in Herpes Stromal Keratitis in Mice. *Invest Ophthalmol Vis Sci* 57, 1749-1756.

Yun, H., Rowe, A.M., Lathrop, K.L., Harvey, S.A., Hendricks, R.L., 2014. Reversible nerve damage and corneal pathology in murine herpes simplex stromal keratitis. *J Virol* 88, 7870-7880.

- Yun, H., Yee, M.B., Lathrop, K.L., Kinchington, P.R., Hendricks, R.L., St Leger, A.J., 2020. Production of the Cytokine VEGF-A by CD4(+) T and Myeloid Cells Disrupts the Corneal Nerve Landscape and Promotes Herpes Stromal Keratitis. *Immunity* 53, 1050-1062 e1055.
- Yun, H., Yin, X.T., Stuart, P.M., St Leger, A.J., 2022. Sensory Nerve Retraction and Sympathetic Nerve Innervation Contribute to Immunopathology of Murine Recurrent Herpes Stromal Keratitis. *Invest Ophthalmol Vis Sci* 63, 4.
- Zhang, A.C., De Silva, M.E.H., MacIsaac, R.J., Roberts, L., Kamel, J., Craig, J.P., Busija, L., Downie, L.E., 2020a. Omega-3 polyunsaturated fatty acid oral supplements for improving peripheral nerve health: a systematic review and meta-analysis. *Nutr Rev* 78, 323-341.
- Zhang, A.C., Downie, L.E., 2019. Preliminary Validation of a Food Frequency Questionnaire to Assess Long-Chain Omega-3 Fatty Acid Intake in Eye Care Practice. *Nutrients* 11.
- Zhang, M., Chen, J., Luo, L., Xiao, Q., Sun, M., Liu, Z., 2005. Altered corneal nerves in aqueous tear deficiency viewed by in vivo confocal microscopy. *Cornea* 24, 818-824.
- Zhang, M., Zhou, Q., Luo, Y., Nguyen, T., Rosenblatt, M.I., Guaiquil, V.H., 2018a. Semaphorin3A induces nerve regeneration in the adult cornea—a switch from its repulsive role in development. *PLoS One* 13, e0191962.
- Zhang, W., Magadi, S., Li, Z., Smith, C.W., Burns, A.R., 2017. IL-20 promotes epithelial healing of the injured mouse cornea. *Exp Eye Res* 154, 22-29.
- Zhang, Y., Chen, P., Di, G., Qi, X., Zhou, Q., Gao, H., 2018b. Netrin-1 promotes diabetic corneal wound healing through molecular mechanisms mediated via the adenosine 2B receptor. *Sci Rep* 8, 5994.
- Zhang, Y., Gao, N., Wu, L., Lee, P.S.Y., Me, R., Dai, C., Xie, L., Yu, F.X., 2020b. Role of VIP and Sonic Hedgehog Signaling Pathways in Mediating Epithelial Wound Healing, Sensory Nerve Regeneration, and Their Defects in Diabetic Corneas. *Diabetes* 69, 1549-1561.
- Zhang, Y.M., Wu, S.Q., Yao, Y.F., 2013. Long-term comparison of full-bed deep anterior lamellar keratoplasty and penetrating keratoplasty in treating keratoconus. *J Zhejiang Univ Sci B* 14, 438-450.
- Zhang, Z., Hu, X., Qi, X., Di, G., Zhang, Y., Wang, Q., Zhou, Q., 2018c. Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice. *Mol Vis* 24, 274-285.
- Zhivov, A., Kraak, R., Bergter, H., Kundt, G., Beck, R., Guthoff, R.F., 2010. Influence of benzalkonium chloride on langerhans cells in corneal epithelium and development of dry eye in healthy volunteers. *Curr Eye Res* 35, 762-769.
- Zhivov, A., Stave, J., Vollmar, B., Guthoff, R., 2005. In vivo confocal microscopic evaluation of Langerhans cell density and distribution in the normal human corneal epithelium. *Graefes Arch Clin Exp Ophthalmol* 243, 1056-1061.
- Zhou, Z., Barrett, R.P., McClellan, S.A., Zhang, Y., Szliter, E.A., van Rooijen, N., Hazlett, L.D., 2008. Substance P delays apoptosis, enhancing keratitis after *Pseudomonas aeruginosa* infection. *Invest Ophthalmol Vis Sci* 49, 4458-4467.