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Understanding clinical and immunological features associated with Pseudomonas and Staphylococcus keratitis

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Abstract

Pseudomonas aeruginosa and *Staphylococcus aureus* are the two dominant Gram-negative and -positive species, respectively, isolated from patients with contact lens-related bacterial keratitis. The clinical features of bacterial keratitis vary, such that timely differential diagnosis can be challenging, which may cause a delay in diagnosis resulting in poorer outcome. This review aims to explore the current understanding of clinical and immunological features associated with contact lens-related *P. aeruginosa* and *S. aureus* keratitis based on currently available evidence.

Firstly, the review characterises contact lens-related *P. aeruginosa* and *S. aureus* keratitis, based on clinical features and prognostic factors. Secondly, the review describes the primary immune response associated with a bacterial infection in *in-vivo* non-scratch contact lens-wearing animal models, colonised by bacteria on contact lens and topical administration of bacteria on the cornea. Finally, the review discusses the role of macrophage inflammatory protein-2 (MIP-2) and intercellular adhesion molecule (ICAM-1) in neutrophil recruitment

based on both *in-vivo* scratch models of bacterial keratitis and bacterial challenged in cell culture models.

1. Background

Contact lens wear (CLW) is a significant risk factor associated with bacterial keratitis, which accounts for 22-65% of cases of bacterial keratitis in hospital or casualty-based studies [1-8]. Bacteria is present in 69-95% of the culture-positive cases of contact lens-related microbial keratitis [3, 9-19]. *Pseudomonas aeruginosa* [3, 9, 10, 13, 19-22] and *Staphylococcus aureus* [2, 9-13, 16, 19, 23, 24] are the most frequently isolated Gram-negative and -positive species from contact lens-related microbial keratitis, respectively. A healthy and intact cornea is highly resistant to invading pathogens in that it can withstand challenges from potentially pathogenic microbes.

Animal models of infection have also contributed to the understanding of the pathophysiology of bacterial keratitis. In the mouse contact lens-wearing scratch models, introduction of a large inoculum of virulent microbes in both contact lens and topical application can result in corneal infection [25-27]. During early bacterial infection in mice, neutrophils migrate and infiltrate into the site of infection from perilimbal circulation as a precursor to the pathophysiology of acute-stage bacterial keratitis [28-30]. Expression of chemokines, such as macrophage inflammatory protein-2 (MIP-2), C-X-C ligand-1 (CXCL1), C-X-C ligand-2 (CXCL2), and intercellular adhesion molecule-1 (ICAM-1) facilitates neutrophil recruitment and modulates the activity of immune cells (e.g., neutrophils, macrophages, dendritic cells, and T-cells) in the cornea [28-32].

The analysis of *in-vitro* corneal cell models has provided a foundation for reporting pathology of bacterial keratitis in humans. For example, *P. aeruginosa* challenged human corneal epithelial cells (HCEC), and human corneal fibroblasts (HCF) express inflammatory mediators such as interleukin-6 (IL-6), interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α),

interleukin-8 (IL-8), intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1(MCP-1) [33-40].

The present review has delineated clinical features of *P. aeruginosa* and *S. aureus* keratitis and secondly has focused on the role of MIP-2 and ICAM-1, along with other chemokines in bacterial keratitis. The clinical features of contact lens-related peripheral ulcer (CLPU) as a form of sterile corneal inflammation are shown for comparison with infectious keratitis.

2. Clinical features of *Pseudomonas aeruginosa* and *Staphylococcus aureus* keratitis

The diagnosis of bacterial keratitis in patients is based on presenting symptoms, history, presenting risk factors, clinical examination and the smear and culture of the corneal scrape.

2.1 Clinical signs and symptoms

Bacterial keratitis typically presents with sudden and rapid onset of ocular pain, redness, blurred vision, tearing, photophobia, and discharge. Acute pain was the main presenting symptom in 30-44% of cases with contact lens-related microbial keratitis [41-44]. The progression of pain occurred in 14% cases of contact lens-related microbial keratitis [43]. Pain was moderate to severe in approximately nine out of ten cases presenting with acute pain [43]. Likewise, redness was a common presenting symptom in 31% of cases with contact lens-related microbial keratitis [43]. Pain with rapid stromal thinning and descemetocele should immediately generate suspicion for *Pseudomonas* infection [45]. Conversely, indolent ulcers due to *Staphylococcus* spp. may be quiet and less symptomatic [46]. An early stage of peripheral bacterial keratitis may look similar to that of CLPU, which may result in inappropriate or delayed management. CLPU is a sterile, focal and localised inflammatory condition [47, 48]. Pain associated with CLPU is either mild or rare in 50% of cases [47, 49]. Redness is sectorial in CLPU, but it is general and diffuse in bacterial keratitis. CLPU also

rarely displays the inflammatory bystander features that are often seen in bacterial keratitis such as anterior chamber response and lid oedema.

Infiltrates, at the centre or para-centre of the cornea within 4 mm from the centre and with an underlying full-thickness epithelial defect, are more likely to be associated with bacterial keratitis [47, 49, 50]. Bacterial keratitis is more likely with deep and dense infiltrates, with a large epithelial defect (greater than or equal to 2.0 mm in size in the greatest linear dimension) and anterior chamber response (Cells greater than or equal to +1) [51].

The clinical features of *P. aeruginosa* and *S. aureus* keratitis are described in Table 1. *P. aeruginosa* keratitis typically presents with a large epithelial defect with a diffuse, serrated and rapidly necrotising stromal lesion, with a yellow-white appearance (Figure 1) [46, 52]. The lesion could extend to the endothelium in severe forms, ultimately leading to corneal perforation [53]. The other important features of *Pseudomonas* keratitis are a ground-glass appearance and loss of transparency of surrounding corneal stroma. Oka et al. (2015) reported ring abscess in 50% of cases with contact lens-related *Pseudomonas* keratitis [52]. Conversely, *Staphylococcus* keratitis mostly appears to be grey-white, discrete and small abscess-like lesions, with a clear margin, minimal surrounding epithelial oedema, and minimal stromal infiltrates [46, 54, 55]. Long-standing *Staphylococcus* keratitis may develop an intrastromal abscess and may perforate [55].

In a multi-centre study, the average area of ulcers on presentation was larger (9.6 ± 15.7 mm^2) in *Pseudomonas* keratitis than in *Staphylococcus* keratitis (5.9 ± 9.3 mm^2), where one-third of the cases were in contact lens wear [56]. Cheng et al. (1999) recorded a mean ulcer diameter of 3.8 mm (approximate ulcer area, $\pi r^2 = 11.3$ mm^2) in contact lens-related *Pseudomonas* keratitis [14]. Similarly, Hoddenbach et al. (2014) reported the size of stromal infiltrates of 3.7 ± 2.0 mm (approximate ulcer area, $\pi r^2 = 10.7 \pm 3.1$ mm^2) in contact lens-related microbial

keratitis where *P. aeruginosa* was isolated in over 80% of cases [53]. Furthermore, nearly 50% of cases with *Pseudomonas* keratitis developed hypopyon, which was twice as high as cases with *Staphylococcus* keratitis [53, 57, 58]. In contrast, infiltrates in CLPU are mostly 0.1-1.5 mm in size (rarely exceeding 2.0mm), in the periphery or midperiphery of the cornea, with secondary break-down of the overlying epithelium (Table 1). The lesion in CLPU is self-limiting and rarely extends deeper than the anterior stroma, and 25% of cases have a trace anterior chamber reaction only [47, 49, 50].

2.2 Prognosis

2.2.1 Visual acuity

Vision loss (less than 6/12 or at least two lines of best-corrected visual acuity) was observed in 14 - 29% cases of contact lens-related microbial keratitis [11, 17, 24, 59, 60]. In a retrospective review, bivariate analyses of severity and vision loss indicated delaying treatment by 49-72 hours (52.9%) was more likely to be associated with a poor visual outcome [11]. In contrast, traumatic keratitis (70%), contact lens-related keratitis (73.9%), and culture-negative cases (65.8%) resulted in a better visual outcome [11, 59]. Multivariate analysis indicated that a poor visual outcome in contact lens-related microbial keratitis was associated with severe keratitis [visual acuity <6/60; odds ratio (OR) = 4.3, confidence interval (CI) = 1.3-6.9], ocular surface disease (OR =4.1, CI = 1.8-9.5) and older age (>50 years, OR = 3.0, CI = 1.3-6.9) [59]. In a population-based study of contact lens-related microbial keratitis, a higher risk of vision loss was associated with disease caused by environmental pathogens (OR = 10.8, CI = 5.3-22.0), delaying treatment by 12 hours (OR = 2.4, CI = 1.0-5.4) and remoteness to care (OR = 2.8, CI = 1.1-7.4) [17]. In the study, *Pseudomonas* spp. was cultured in 56% of cases [17].

In a randomised multi-centre clinical trial of adjunctive corticosteroid treatment of non-contact lens-related *Pseudomonas* keratitis in India and the USA, presenting best-corrected visual

acuity (BCVA) was worse in *Pseudomonas* keratitis than in other types of bacterial keratitis by an average difference of 2.5 lines [61]. However, visual acuity improved in treated *Pseudomonas* keratitis to a similar extent as in other types of bacterial keratitis of similar severity [61]. In a retrospective review of medical records in Australia, *Pseudomonas* keratitis showed even a good final visual outcome (better than 6/12) in 54.5% compared to 30.8% in *Staphylococcus* keratitis where 22% of subjects were contact lens wearers [59]. Overall final visual acuity was recorded as poor (less than 6/60) in 38.5% cases of *Staphylococcus* keratitis and in 27.3% cases of *Pseudomonas* keratitis [59]. In a retrospective study including both contact lens wearers and non-wearers in Taiwan, poor final BCVA in *Pseudomonas* keratitis was associated with a hypopyon, large and deep infiltration after adjusting for age, sex and contact lens wear (CLW) [58]. Conversely, poor final visual acuity in *Staphylococcus* keratitis was associated with advanced age and poor initial visual acuity in another hospital-based retrospective study in Taiwan, where a small proportion of subjects were contact lens wearers (15.2%) [57]. However, a better analysis would be looking at CLW and non-CLW related bacterial keratitis separately to compare the outcome.

2.2.2 Corneal healing and scarring

The outcome of bacterial keratitis varies depending on the severity of the infection and the causative organism [13, 17]. Mild cases are generally treated with topical monotherapy, while complicated cases might require combination and high dose therapy and in-patient care [7, 57, 61-64]. In contact lens-related bacterial keratitis, complete corneal epithelisation occurred after one-week (range = 2-77 days), where *P. aeruginosa* was the primary isolate in 55% of cases [3]. In a multi-centre study in the UK, Kaye et al. (2010) found that both treatment and healing times were similar between non-contact lens-related *Pseudomonas* and *Staphylococcus* keratitis (Table 1) [56]. Alternatively, in terms of healing time per unit ulcer area, *Staphylococcus* keratitis required a slightly longer time than did *Pseudomonas* keratitis [56].

However, Kaye et al. (2010) documented that *Pseudomonas* keratitis developed larger (7.2 ± 15.2 mm^2) corneal scars than did *Staphylococcus* keratitis (3.8 ± 7.8 mm^2) [56]. In a study by Shen et al. (2015) where 57% of cases were contact lens wearers, both females and contact lens wearers exhibited rapid re-epithelisation in *Pseudomonas* keratitis, whereas large and deep infiltration was associated with delayed healing [58]. Compared with microbial keratitis, CLPU resolves rapidly upon contact lens discontinuation, although it may require a course of prophylactic topical antibiotics and steroids [65]. Without treatment, 21% of cases resolve in seven days, and the majority had resolved in 3 weeks [47].

In summary, contact lens-related *Pseudomonas* keratitis occurs mainly in young individuals without comorbidities or other ocular surface diseases. Although *Pseudomonas* keratitis presents with a large ulcer size and severe infection, corneal re-epithelialisation is more rapid and visual outcome is better compared with *Staphylococcus* keratitis. Non-contact lens-related *Pseudomonas* keratitis is associated with a larger scar than non-contact lens-related *Staphylococcus* keratitis.

3. Pathology of contact lens-related bacterial keratitis in animal models

Animal models have provided invaluable insight into the host-response in contact lens-related bacterial keratitis [25, 27, 72]. Chiefly, two variants of mice (C57BL/6 and BALB/c) have commonly been compared with wild-type mice in both scratch and non-scratch models of both contact lens-related and non-contact lens-related bacterial keratitis. C57BL/6 (or B6) mice are common inbred strains of laboratory mice and are susceptible Th1 responders while BALB/c mice are immunodeficient laboratory-bred strains of house mice, which are susceptible Th2 responders [73]. In *Pseudomonas* keratitis in mice, Th1-mediated corneal inflammation can clear bacteria more efficiently than the Th2 response, but this is associated with increased disease severity and damage to the corneal tissues [74]. The two main sub-types of T

lymphocytes are distinguished by the presence of cell surface molecules called cluster of differentiation (CD)4 and CD8. T lymphocytes with CD4 are called T helper cells (Th) and are the most prolific cytokine producers. Cytokines are the hormonal messengers responsible for most of the biological effects in the immune system. T helper cells may be further divided into Th1 and Th2 and the cytokines they produce are known as Th-1 type cytokines and Th-2 type cytokines. T lymphocytes are a major source of cytokines and bear antigen-specific receptors on their cell surface that enables recognition of foreign pathogens [75]. Therefore, Th1 is associated with an excessive pro-inflammatory response, whereas Th2 is associated with anti-inflammatory response [75]. The corresponding wild-type mice are sterile strains of a typical phenotype found in nature [25, 27, 30, 40, 74, 76-81]. In non-scratch animal models of contact lens-related bacterial keratitis, New Zealand white rabbits, [82, 83] and female Lewis rats (susceptible to infection) have also been used [72, 82, 84].

In a non-scratch extended CLW model in the rabbit, sterile corneal infiltrates were seen after three weeks of lens wear upon topical application of *S. aureus* cell suspension (strain 031; isolated from a patient experiencing CLPU) [83]. Further, severe keratitis occurring within 24 hours was more likely to be associated with hydrogel contact lens colonised with *S. aureus* 8325-4 than the lens colonised with *S. aureus* DU1090, indicating the likely role of α-toxin. *S. aureus* 8325-4 is an α-toxin positive parent strain and DU1090 is its isogenic (i.e. having the same or closely similar genes) α-toxin negative mutant. α-toxin has a proven virulence factor in several animal infection models and is essential for infections that disrupt epithelial barriers such as in the cornea [84-86]. Therefore, *S. aureus*-associated α-toxin causes epithelial cell lysis exposing the underlying stroma and increasing neutrophil density [84-86]. β-toxin is a form of sphingomyelinase and is toxic to a variety of cells including fibroblasts, leukocytes and macrophages. Susceptible cells are subject to lysis of exposed sphingomyelin on their

membrane surfaces [87-90]. However, *S. aureus*-associated β-toxin was less lethal to stromal fibroblasts though it caused some corneal inflammation in a mouse model [87].

The pathology of contact lens-related *Pseudomonas* keratitis in non-scratch animal models is summarised in Table 2. Silicone hydrogel (SiH) contact lens alone, without introduction of bacteria, caused no visible corneal pathology, and corneal clarity was similar to non-lens wearing mice [25]. Moreover, CLW for more than two weeks did not induce cytokine mRNAs [e.g., interleukin-1α (IL-1α), interleukin-1 receptor antagonist (IL-1RA), transforming growth factor- β (TGF-β) and macrophage migration inhibitory factor (MIF) mRNAs] in the cornea in the absence of bacterial challenge [91]. Cytokine mRNAs are the gene transcriptions of cytokines, which regulate the expression of cytokines. However, an early immune response could be seen with CLW in corneas colonised with *P. aeruginosa* (PAO1-GFP). Strain PAO1-GFP is a mutant of the reference strain PAO1 (poorly virulent laboratory reference) transformed with plasmid pSMC2 expression enhanced Green Fluorescent Protein. A SiH lens colonised with *P. aeruginosa* caused keratitis in 9% of mice as early as 24 hours which increased to 55% after 11 days of extended CLW [25].

Pseudomonas keratitis is described in terms of neutrophil recruitment via an interleukin-1 receptor (IL-1R) and MyD88 (Myeloid differentiation primary response 88) and the type III secretion systems (T3SSs). Metruccio et al. (2019) reported neutrophil recruitment through an IL-1R and MyD88 protein-dependent manner following five days of CLW colonised with *P. aeruginosa* [25]. IL-1R is a cytokine receptor which binds interleukin 1 (IL-1). Two forms of the receptor exist. The type I receptor is primarily responsible for transmitting the inflammatory effects of IL-1, while type II receptors may act as a suppressor of IL-1 activity by competing for IL-1 binding [92]. MyD88 is a protein that, in humans, is encoded by the MyD88 gene. The MyD88 gene provides instructions for making a protein involved in signalling within immune cells. The MyD88 protein acts as an adapter, connecting proteins that receive signals from

outside the cell to the proteins that relay signals inside the cell [93]. Moreover, the T3SSs are significant virulent factors in the pathogenesis of *Pseudomonas* keratitis. T3SSs are complex bacterial structures that provide gram-negative pathogens with a unique virulence mechanism enabling them to inject bacterial effector proteins directly into the host cell cytoplasm. The activity of the T3SSs correlates closely with infection progression and outcome, both in animal models and in human infection [94]. The genotype of *P. aeruginosa* strains is categorised as either cytotoxic or invasive based on the type of T3SSs exotoxin secretion [58]. Cytotoxic strains (exoU+ genotype) predominate in contact lens-related keratitis, whereas invasive strains (exoS+ genotype) predominate in non-contact lens-related keratitis [58, 95, 96]. However, exoU+ genotype was absent in the majority of contact lens-related isolates from Australia in a recent study [97]. The strains with genotype exoS can also increase their survival by detoxifying reactive oxygen species (ROS) produced by neutrophils [98]. In a clinical and laboratory-based study of *Pseudomonas* keratitis, cytotoxic strains caused less severe keratitis with smaller infiltrates and more rapid re-epithelisation than did invasive strains [58]. Similarly, Szliter et al. (2006) examined the rapid host response to *P. aeruginosa* 19660 (a laboratory strain known to produce severe keratitis in experimentally infected mice) in female Lewis rats fitted with Lotrafilcon A contact lenses [72]. Neutrophil levels significantly increased in the experimentally challenged cornea, in addition to upregulation of IL-1β and IL-6 mRNAs [72]. Further, the severity of *Pseudomonas* keratitis in contact lens-fitted mice was associated with the level of inflammatory proteins (TNF-α, IL-1β and IL-6) as well as neutrophil count in norepinephrine treated B6 mice more than in their controls. Norepinephrine is a neurotransmitter which is released during a stress response. Application of norepinephrine increased the severity of keratitis [27]. It is clear that in these animal studies that the CL-wearing eye can remain free of pathology in the absence of bacterial challenge, but the CL-

wearing eye challenged with bacteria shows an early immune response as the first step in the pathogenesis of *Pseudomonas* infection.

4. Neutrophils are primary immune mediators in early bacterial keratitis in mice models

Corneal infiltrates in bacterial keratitis are aggregations of neutrophils which accumulate to clear invading pathogens and their antigens. Principally, intercellular communication between infiltrating leukocytes, corneal tissues, and the limbal vascular endothelium determines neutrophil recruitment [74]. In animal studies, rapid neutrophil recruitment drives the host's innate immune response by activating Th1 cells at the site of infection [74, 99-101]. Prolonged neutrophil recruitment may trigger the release of extracellular lysosomal enzymes, which can cause further damage to the cornea [74].

CD4+T cells and macrophages regulate neutrophil recruitment in *P. aeruginosa* infected B6 and BALB/c mice cornea, respectively [74]. CD4+ cells are a type of T helper cells (Th cells) that play particularly an important role in the adaptive immune system. In B6 mice, CD4+T cell-mediated neutrophil recruitment was associated with the severity of *Pseudomonas* keratitis [78, 79]. Macrophage regulated neutrophil recruitment occurred more in BALB/c mice than in B6 mice though the severity of *Pseudomonas* keratitis was comparable between the two strains of mice [30, 76]. In mouse scratch model, *P. aeruginosa* (strains expressing exoS) keratitis demonstrated massive neutrophil recruitment underlying the area of bacterial aggregation in the cornea at 24 hours post-infection. The neutrophils inhibited biofilm formation and spreading of *P. aeruginosa* and confined *P. aeruginosa* out of the cornea surface by forming neutrophil extracellular traps (NETs) [102]. NETs thus protect against infection. NETs formation is triggered by innate immune receptors through downstream intracellular mediators. NETosis is induced in response to microbial cues and endogenous danger signals and must be

tightly regulated in order to prevent excessive tissue damage during acute inflammation [103]. Consequently, the NETs can degrade corneal collagens and cause severe ulceration [102].

Human CXC chemotactic cytokines [e.g., C-X-C ligand-2 (CXCL2), IL-8) behave as potent chemotactic factors for neutrophil recruitment [74]. CXC chemotactic cytokines such as CXCL2 are neutrophil chemo-attractants that produce several responses that are essential for antimicrobial host defence, namely shape change, directional migration and exocytosis. This is a form of active transport for movement of molecules out of a cell by a process of vesicles fusing with the plasma membrane and releasing their contents to the outside of the cell. Exocytosis is a complex response involving the release of enzymes and other soluble proteins from several subcellular storage compartments and the re-modelling of the plasma membrane by fusion with subcellular membranes [104]. Macrophage-inflammatory protein 2 (MIP-2) is a chemokine (C-X-C) ligand 2 (CXCL2) protein and is the mouse homologue of the human IL-8 [74]. MIP-2 is a major CXC chemokine involved in the migration of polymorphonuclear neutrophils (PMNs) to sites of inflammation. PMNs migrate from the tear film and from the limbal and iridial vasculature into the avascular cornea. In mouse models of corneal infection, MIP-2 mediates neutrophil recruitment in the cornea, causing a cascade of inflammatory events. Similarly, Intercellular adhesion molecule 1 [ICAM-1, also known as CD54 (Cluster of Differentiation 54)] is a key molecule for neutrophil recruitment into infected tissue. Bacterial endotoxin stimulates human corneal fibroblasts to express ICAM-1 to mediate recruitment of inflammatory cells, including neutrophils and to initiate the pathogenesis of bacterial keratitis [37, 38].

4.1 Macrophage inflammatory protein-2 (MIP-2) is essential for active neutrophil recruitment in bacterial keratitis in mice model

Macrophage Inflammatory Protein 2 (MIP-2), also known as CXCL2, is a potent neutrophil chemoattractant (Figure 2A), which is secreted by monocytes, macrophages and epithelial cells, is activated through the p38 mitogen-activated-protein-kinase-dependent signalling pathway and binds to the receptor CXCR2 (C-X-C chemokine receptor-2) [105]. The CXCR2 binds several different chemokines to trigger its function. It is expressed on immune cells including neutrophils, mast cells, monocytes and macrophages. The main function of CXC chemokines is to attract mononuclear cells to sites of chronic inflammation [106]. Chidambaram et al. (2017) reported that the CXCL2 gene was highly upregulated in the culture-positive cases of late-stage bacterial keratitis in human [107].

MIP-2 was notably upregulated in B6 mouse cornea to a greater extent than in BALB/c and wild-type mice in *in-vivo* studies of corneal infection [30, 76]. Kernacki et al. (2000) found *P. aeruginosa* (strain 19660) keratitis caused significant neutrophil recruitment in B6 mice between five- and seven-days post-infection. Further, increased MIP-2 level was associated with increased neutrophil recruitment and more severe corneal infection, which resulted in corneal perforation in all mice at seven days post-infection [76]. Xue et al. (2003) further demonstrated prolonged-expression of MIP-2 in BALB/c mice cornea was associated with the severity of corneal inflammation and increased neutrophil recruitment, irrespective of bacterial load [81]. In the presence of bacterial endotoxin, MIP-2 also promoted neutrophil recruitment in the corneal stroma of BALB/c and C3H/HeN mice [normal LPS (lipopolysaccharide) responsive variants) [40]. LPS, also known as endotoxin, is a macromolecule consisting of a lipid and a polysaccharide composed of O-antigen oligosaccharide side chain in the outer membrane of Gram-negative bacteria.

The recovery of inflammatory mediators from gene knockout (gko) and genetically modified mouse corneas during infection, suggested that many receptors [CXCR2, TLR4 (toll-like receptor-4), TLR9 (toll-like receptor-9)] along with IL-1β protein could mediate the release

317 and activity of MIP-2 [30, 40, 81, 101, 108-110]. For instance, CXCR2 receptors were
318 necessary to bind MIP-2 for effective neutrophil chemotaxis and extravasation into the site of
319 *S. aureus* and *P. aeruginosa* keratitis as noted in mice studies (Figure 2.A) [101, 108]. Despite
320 the high level of MIP-2 following corneal infection in CXCR2 knock out mice, neutrophils
321 were confined to the perilimbal region. The lack of CXCR2 disrupted the MIP-2 driven
322 neutrophil migration to the site of infection, impairing bacterial clearance and causing keratitis
323 to progress to perforation [101, 108]. Furthermore, the C3H/HeJ (TLR4 gene mutated, LPS
324 non-responsive) mice cornea could not produce MIP-2 and could not exhibit neutrophil
325 recruitment in response to *P. aeruginosa* endotoxin [40]. Alternatively, IL-1β appeared to be a
326 key cytokine associated with expression of MIP-2 in *P. aeruginosa* infection in both B6 and
327 BALB/c mice corneas [30, 81]. Prolonged-expression of IL-1β could be a precursor to the
328 overexpression of MIP-2, which augmented neutrophil recruitment to the site of infection,
329 causing corneal damage [30]. The association between MIP-2 and IL-1β was demonstrated in
330 the infected cornea of TLR9 siRNA (silencing RNA) treated B6 mice [109]. In TLR9 siRNA-
331 treated B6 mice, the reduced level of IL-1β was associated with the reduced MIP-2 level and
332 the neutrophil counts in *in-vivo* [109]. Recently, in the TNF-α induced protein 8-like-2 (TIPE2)
333 knockout mouse model, increased susceptibility to *Pseudomonas* keratitis has been associated
334 with upregulation of MIP-2, along with increased neutrophil recruitment and other
335 inflammatory mediators *in-vivo*, and the study indicated that TIPE2 mediated MIP-2 could
336 regulate neutrophil recruitment [111]. These findings in gene-knockout animals suggest that
337 the pathology of corneal infection is in large part due to the degree of the host response, which
338 may be beyond that required for infection control and that this balance is important in
339 determining the outcome in bacterial keratitis.

Overall, MIP-2 was critical for active neutrophil recruitment, which was principally mediated by receptors present in the mice cornea. Sustained expression of MIP-2 in bacterial keratitis can increase disease severity even in the resistant strain of mice.

4.2 Intercellular adhesion molecule (ICAM-1) expression is associated with both neutrophil recruitment and the severity of bacterial keratitis

Intercellular adhesion molecule-1 (ICAM-1, CD54) is a transmembrane glycoprotein, functioning as an adhesion molecule in a variety of biological situations. ICAM-1 belongs to the immunoglobulin superfamily (IgSF), which is a large protein superfamily of cell surface and soluble proteins that is involved in the recognition, binding, or adhesion processes of cells [114]. ICAM-1 is typically expressed on immune cells. ICAM-1 was expressed weakly on keratocytes, corneal endothelial cells and perilimbal vascular endothelial cells of healthy human corneoscleral specimens and cultured human corneal endothelial cells [115, 116]. ICAM-1 was also expressed in the culture of human corneal epithelial cells, keratocytes and endothelial cells during bacterial challenge [37-39, 117]. ICAM-1 was further associated with a higher neutrophil density in human corneal stroma than in human corneal epithelium in bacterial keratitis [36]. In the corneal fibroblasts, LPS-induced release of ICAM-1 activated the NF-kB (nuclear factor kappa light chain enhancer of activated B cells) pathway (Figure 2.B) [38, 118, 119]. NF-κB is a family of transcription factors that regulate many important cellular behaviours, in particular, inflammatory responses, cellular growth and apoptosis. NF-kB also regulates innate immune response and expression of proinflammatory genes including cytokines, chemokines and adhesion molecules [38, 120].

ICAM-1 is a key mediator of acute ocular inflammation in *P. aeruginosa* infection in the mouse cornea, contributing to neutrophil recruitment and increasing disease severity. ICAM-1 deficient mice infected with *P. aeruginosa* (ATCC 19660) demonstrated less severe keratitis

than wild-type mice, which in wild-type animals presented with a relatively clear central cornea, fewer inflammatory cells and comparable expression of IL-1β and TNF-α [121]. Mouse age also affected the level of ICAM-1 in *P. aeruginosa* infection. There was increased immunostaining for ICAM-1 of the corneal epithelium, keratocytes and endothelium in young mice (6-8 weeks old) than in aged mice (1.5–2.0 years old). Conversely, aged mice had significantly less neutrophil recruitment in the corneal stroma, along with less severe corneal pathology [122]. Similarly, Interleukin-6 (IL-6) dependent expression of ICAM-1 could effectively recruit neutrophils and could limit the severity of *P. aeruginosa* keratitis in B6 mice (Figures 2.A) [112]. IL-6 is a pleiotropic, pro-inflammatory cytokine produced by a variety of cell types, including lymphocytes, monocytes, and fibroblasts [123]. In IL-6 gko B6 mice, *P.aeruginosa* corneal infection caused downregulation of ICAM-1 in the corneal epithelium after 12 hours of infection. Following treatment with IL-6, the level of ICAM-1 increased in the epithelium and the stromal keratocytes adjacent to the corneal endothelium [112]. Further, CXCR2 was implicated in the binding and signalling of ICAM-1, in addition to binding and signalling of MIP-2 in mice cornea (Figure 2.A). Similarly, CXCR2 knockout mice were unresponsive to ICAM-1 in *S. aureus* keratitis and showed delayed neutrophil recruitment (Figure 2.A). [101] Therefore, early appropriate neutrophil recruitment is necessary for better resolution of corneal infection.

In *Pseudomonas* LPS-stimulated human corneal fibroblasts (HCF), the expression of ICAM-1 was associated with CD14 (cluster of differentiation 14), TLR4 and MD-2 (myeloid differentiation-2) gene expressions. CD14, TLR4 and MD-2 usually form a receptor complex in response to bacterial antigens to trigger inflammatory cell recruitment through the expression of chemokines and adhesion molecules (Figure 2.B) [37]. Studies showed that LPS-binding proteins (LBP) and CD14 could mediate the expression of ICAM-1, along with other chemokines (IL-8 and MCP-1) in LPS-stimulated HCF and could mediate translocation of NF-

kB [37, 117]. Similarly, *S. aureus* lipoprotein-stimulated telomerase-immortalised human corneal epithelial cells could mediate TLR2 (toll-like receptor-2) to express ICAM-1, IL-6, and IL-8 and to activate NF-kB signalling pathways [39]. Therefore, ICAM-1 was identified as one of the key mediators of neutrophil recruitment in human corneal tissues in bacterial corneal infection.

In conclusion, upregulation of ICAM-1 is associated with increased neutrophil recruitment and disease severity in mouse cornea *in-vivo* and culture of human corneal epithelial cells and stromal fibroblasts *in-vitro*. However, early controlled expression of ICAM-1 can recruit neutrophils effectively to control early corneal infection.

4.3 Additional CXC chemokines associated with *Pseudomonas* and *Staphylococcus* corneal infection

CXC chemokines are a group of specific signalling proteins, called cytokines, secreted by cells at sites of infection and inflammation. Chemokines have been classified into four main types: CXC, CC, CX3C and XC. All of these proteins exert their biological effects by interacting with G protein-linked transmembrane receptors called chemokine receptors that are selectively found on the surfaces of their target cells. CXC chemokine receptors are integral membrane proteins that specifically bind and respond to cytokines of the CXC chemokine family. There are currently seven known CXC chemokine receptors in mammals, named CXCR1 to CXCR7 [124]. A number of CXC chemokines are associated with *P. aeruginosa* and *S. aureus* corneal infection (Table 3). IL-8 was frequently upregulated in *P. aeruginosa* and *S. aureus* challenge of cultured human corneal epithelial cells (HCEC) and human corneal stromal fibroblasts (HCF) [34, 125, 126]. *Pseudomonas*-associated LPS also upregulated IL-8 expression in primary HCF [38, 127]. CD14 and LBP mediated the secretion of IL-8 in a dose-dependent manner in HCEC challenged with *Pseudomonas*-associated LPS [128]. Further, NF-kB and IL-1β facilitated IL-8 expression in *Pseudomonas* challenged HCEC [34, 129]. Similarly, the

quorum-sensing signalling molecule, n-(3-oxododecanoyl)-l-homoserine lactone stimulated IL-8 expression in HCEC [126]. However, the antimicrobial peptide cathelicidin diminished IL-8 expression in a dose-dependent manner in the cultured HCF [38]. Furthermore, *Staphylococcus* challenge of HCEC and stromal cells of donor corneas caused expression of IL-8 mRNA [130, 131]. Early upregulation of IL-8 was also found in rabbit cornea challenged with UV-killed *S. aureus in-vitro*, which could indicate the presence of an immediate immune response to the bacterial infection in rabbit cornea [130]. Further, *Staphylococcus* infection of HCEC significantly increased C-C chemokine ligand 20 (CCL20) mRNA expression independent of TLR2 and Nucleotide-binding oligomerisation domain-containing protein-2 (NOD2) [131]. CCL20 is strongly chemotactic for lymphocytes and weakly attracts neutrophils. Likewise, NOD2 plays an important role in the immune system. NOD2 recognises bacterial molecules (peptidoglycans) and stimulates an immune reaction. In bacterial corneal infection, TLR2 acts as a sensor for Gram-positive bacteria and their lipoproteins, whereas it suppresses *Pseudomonas*-associated LPS-induced immune response mediated by TLR4 [34, 38, 39]. Toll-like receptor 2 (TLR2) is a transmembrane surface protein that in humans is encoded by the TLR2 gene. It plays a fundamental role in pathogen recognition and activation of innate immunity. TLR2 recognises foreign substances and transmits signals to certain cells of the immune system. Toll-like receptor 4 (TLR 4) is a protein that in humans is encoded by the TLR4 gene. TLR4 is another transmembrane protein member of the toll-like receptor family [132].

During *in-vitro Pseudomonas* infection of the B6 mouse cornea, CXCL1 and CXCL2 were upregulated, which was associated with increased bacterial counts in the cornea [133]. The CXCL1 is a small peptide belonging to the CXC chemokine family that becomes chemotactically active for neutrophils. CXCL2 is a cytokine belonging to the CXC chemokine family that is also called macrophage inflammatory protein 2α (MIP-2α). CXCL2, like related

chemokines, is also a powerful neutrophil chemoattractant. Conversely, *in-vivo Pseudomonas* corneal infection in BALB/c mice downregulated C-C chemokine ligand 2 (CCL2) and C-C chemokine ligand 3 (CCL3) [134]. CCL2 (also known as monocyte chemoattractant protein-1, MCP-1) and CCL3 (also known as macrophage inflammatory protein 1α, MIP-1α) are cytokines belonging to CC chemokine family. Application of anti-CCL2 and anti-CCL3 antibodies reduced the severity of corneal infection, neutrophil recruitment and the level of IL-1β, MIP-2, keratinocyte-derived chemokine (KC, also known as CXCL1) and vascular endothelial growth factor (VEGF) after one to seven days post-infection [134]. Further studies showed upregulation of KC protein during *in-vivo Pseudomonas* corneal infection in B6 and BALB/c mice [32, 81, 113, 135]. IL-1β regulated the activity of KC, which was located in the epithelium and stroma corresponding to neutrophil recruitment [81, 135]. The level of KC was associated with increased angiogenesis in *Pseudomonas* corneal infection [32]. In *Staphylococcus* corneal infection of BALB/c mice, upregulation of KC was associated with ineffective neutrophil recruitment [32]. These results suggest the relevance of the expression and role of IL-8, CXCL1, CXCL2, CCL2, CCL3, CCL20 and KC in *Pseudomonas and Staphylococcus* corneal infection and need for further exploration. Understanding their potential role in early infection could be pivotal in understanding the pathophysiology of corneal infection.

5. Summary and future directions

Contact lens-related bacterial keratitis is rapidly progressing acute clinical condition, which requires urgent diagnosis and treatment. In the early stage, contact lens-related bacterial keratitis can be challenging to differentiate from symptomatic sterile infiltrates like CLPU. Certain features may be more suggestive of a specific causative agent. However, confirmed diagnosis of a causative organism requires culture or molecular techniques from corneal scrapes or corneal biopsy.

P. aeruginosa causes a rapidly progressing keratitis associated with corneal necrosis; hence it warrants urgent management [136, 137]. *Staphylococcus* keratitis can also progress rapidly, maybe sight-threatening and is associated with a delay in wound healing [56, 138]. This review has provided insight into contact lens-related *Pseudomonas* and *Staphylococcus* keratitis. Although some clinical features are common between contact lens-related and non-contact lens-related bacterial keratitis, the host response to bacterial virulence factors which underpin disease progression, needs further exploration. TLR4 binds LPS in *P. aeruginosa*. Likewise, TLR2 acts as a sensor of *S. aureus* and its lipoproteins and peptidoglycan [34, 39]. Similarly, CXCR2 signals the presence of *P. aeruginosa* and *S. aureus* corneal infection and facilitates the activity of MIP-2 and ICAM-1 whereas TLR9 is active in *P. aeruginosa* corneal infection and is implicated in corneal opacification and perforation [101, 108, 109]. The difference in the pathology of *Pseudomonas* and *Staphylococcus* corneal infection may also depend on the interaction between bacterial virulence factors and the host immune factors.

Bacterial keratitis leading to corneal scarring is one of the leading causes of corneal blindness [139]. Many bacteria produce tissue-dissolving enzymes and proteins [37-40, 117]. Animal models and gko mutants have been used to explore the host response to bacterial keratitis. In non-scratch models in mice and rabbits, CLW along with bacteria, either in the form of a colonised contact lens or topical administration is required for corneal infection (Table 2). In contact lens-related *P. aeruginosa* keratitis, cytotoxic strains are more common and have better clinical prognosis than do invasive strains [58]. Likewise, *S. aureus*-associated α-toxin is identified as more lethal than β-toxin to corneal tissues [87].

Some *in-vitro* studies of bacterial challenge of HCECs and stromal fibroblasts are available, and they could also provide insight into the underlying pathogenesis of bacterial keratitis. Molecular investigation of bacterial keratitis can further identify primary inflammatory cells and proteins involved in the pathogenesis. In this present review, MIP-2 and ICAM-1 have

been explored based on evidence that molecules are essential for neutrophil recruitment to initiate a primary immune response in corneal infection. The literature has suggested that active neutrophil recruitment is necessary to hasten bacterial clearance and improve resolution. Conversely, the overwhelming host response may cause excessive neutrophil recruitment and subsequent tissue damage resulting in corneal perforation or scarring. Therefore, MIP-2 and ICAM-1 could be potential markers of severity and pathogenesis of early *Pseudomonas and Staphylococcus* corneal infection. Further molecular and biochemical studies are necessary to elucidate the host response to invading pathogens and explore the role of MIP-2 and ICAM-1.

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Tables

Table 1. Clinical features of *Pseudomonas* and *Staphylococcus* keratitis and contact lens-related peripheral ulcer

Table 2. Pathology of contact lens-related bacterial corneal infection in non-scratch animal models

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Figures

Figure 1. *Pseudomonas aeruginosa* keratitis showing diffuse corneal infiltrates extending from the centre to midperiphery of the cornea (6.5 mm diameter), overlying epithelial defect (diameter in the longest meridian = 2.9 mm), with an irregular margin and perilimbal and diffuse injection.

Figure 2.A: A schematic network of macrophage inflammatory protein-2 (MIP-2) and intercellular adhesion molecule-1 (ICAM-1) expression in mouse scratch models of bacterial keratitis and the subsequent recruitment of neutrophils to the cornea. [40, 76, 81, 101, 108, 109, 112, 113]

MIP-1α = macrophage inflammatory protein-1α, rMIP-1α = recombinant-IMP-1α, TLR = toll-like receptor, CXCR2 = CXC chemokine receptor 2, CXCL2 = CXC chemokine ligand-2, IL-

1β = interleukin-1β, ICAM-1 = intracellular adhesion molecule-1, MIP-2 = macrophage inflammatory protein-2, IL-6 = interleukin-6, KC = keratinocyte-derived chemokine (also known as CXC Ligand-1, CXCL1)

#gene knockout models [CXCR2 gko, IL-6 gko, HMGB1 (high mobility group box protein-1) gko and ICAM-1 gko], §genetic modification [TLR4 (toll-like receptor-4) mutation and TLR9 siRNA(toll-like receptor-9 silencing RNA)], *application of rMIP-1α

B6 mice = C57BL/6 strains of susceptible Th1 responding mice, BALB/c mice = strains of susceptible Th2 responding mice

P. aeruginosa included strains19660 (cytotoxic laboratory strain), strains 6294 (invasive strains), strains 6206 (cytotoxic strains), *S. aureus* 38 (a clinical isolate from human corneal ulcer)

Figure 2.B A schematic network of intercellular adhesion molecule-1 (ICAM-1) expression in human corneal tissues in response to bacterial lipopolysaccharide (LPS) in the cell culture models. [36-39, 118]

(a) human telomerase-immortalised corneal epithelial cell line (HUCL), (b) primary human corneal fibroblasts.

LPS = lipopolysaccharide; LBP = LPS binding protein; TLR2 = toll-like receptor-2, TLR4 = toll-like receptor-4, CD14 = cluster of differentiation 14, MD-2 = myeloid differentiation-2, ICAM-1 = intracellular adhesion molecule-1, IL-8 = interleukin-8, MCP-1 = monocyte chemotactic proteins-1, NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells.

(1) The surface of human corneal fibroblasts binds a bacterial endotoxin with LBP, (2 & 3) subsequently, bacterial protein is presented to the receptor complex such as CD14, MD-2 and TLR4. The events trigger inflammatory cell infiltration through the expression of chemokines and adhesion molecules (e.g., ICAM-1 and IL-8) on the cell surface. Therefore, activation of corneal epithelium and fibroblasts could be necessary for the pathogenesis of bacterial keratitis

Table 1. Clinical features of *Pseudomonas* and *Staphylococcus* keratitis and contact lens-related peripheral ulcer

Characteristics	***Pseudomonas* keratitis**	***Staphylococcus* keratitis**	**CLPU**	**References**
†Proportion of cases	Median 27 % (range 2 - 69 %) of total cases of contact lens-related microbial keratitis‡	Median 4.0 % (1 - 21 %) of total cases of contact lens-related microbial keratitis‡	9 – 30 % of total cases of noninfective infiltrative events	[2, 5, 9-13, 21, 24, 42, 53, 59, 62, 66, 67]
Demographics				
†Age	Average 25 years (1 - 73); 57% cases - aged between 25-44 years.	*Average 56 (1 - 83) years	Average 25.1 ± 9.4 years for all types of noninfective infiltrates.	[16, 21, 24, 52, 53, 57, 68, 69]
†Gender	Median 62% female (55-77%)	46% females§	63% females (for all types of noninfective infiltrates)	[16, 21, 52, 68]
Symptoms				
#Pain	Usually severe	Mostly moderate, but could be severe	Usually mild in nearly half of cases.	[46, 47, 49]
#Redness/ Injection	Severe and generalised	Moderate to severe and generalised	Mild and localised, seldom exceeds a quadrant	[47, 49]
#Photophobia	Severe	Moderate	Absent to mild	[46, 49]
#Watering	Intense	Mild to moderate	Mild in nearly half of the cases	[46, 47, 49]
†Progression	Rapid and progressive	Rapid and progressive	Self-limiting	[47, 49, 58]
#Discharge	Intense mucopurulent yellow-greenish exudation	Moderate mucopurulent exudation	Rare	[46]
#Swelling	Present (Severe)	Present (mild to moderate)	Rare	[49]
Clinical Signs				
#Epithelial defect	Full-thickness	Full-thickness	Punctate staining (16.7%) to full-thickness (83.7%)	[47, 49, 50]
# Infiltration	Early-stage - focal infiltrates; advance stage-diffuse and rapidly spreading necrotic lesions	Discrete with a clear margin and small abscess-like lesions	Focal and localised lesion rarely exceeding beyond a quadrant	[46, 47, 50]
#Location	Mostly central and paracentral cornea but can be peripheral as well	Can be central, paracentral and peripheral	Mostly periphery but can be paracentral as well	[47, 57]
#Size	Approximate length of 4.0 mm (area 9.6 ± 15.7 mm^2)	Approximate length of 1.7 mm (area = 5.9 ± 9.3 mm^2; ranges between 2mm and 6mm in 66.4% of cases)	Approximate length of 0.1-1.5 mm (rarely exceed beyond 2.0 mm)	[14, 47, 56, 57]
#Shape	Irregular and diffuse	Irregular and localised	Circular or oval	[49, 50]

#Depth	Anterior to the posterior stroma	Anterior to the mid- stroma	Anterior stroma	[58]
#Density	Yellow white, opaque	Grey-white Opaque	White or grey-white, translucent to opaque	[46, 49]
†Anterior chamber response	Almost always present	Almost always present	Absent or a slight anterior chamber response in 25% of the cases	[47, 49]
†Hypopyon	Hypopyon around 58% of cases	*Hypopyon around 22% of cases	Nevel present	[49, 53, 57, 58]
Prognosis				
#Visual acuity	Worse initially but shows good visual recovery. Overall final vision may be poor due to large corneal scars.	Vision reduced with poor recovery	Usually unaffected	[47, 57, 61]
#Therapy	Need intensive antibiotic therapy	Need intensive antibiotic therapy	Spontaneous healing on discontinuation of CLW; May require prophylactic antibiotics and topical steroids to speed resolution.	[7, 12, 56, 57, 61-65]
#Therapy time (days)	23.6 ± 15.2	21.3 ± 14.6	-	[56]
#Healing time (days)	15.2 ± 16.8	14.6 ± 12.5	7 days (21% of cases); majority resolved in 3 weeks	[47, 56]
*Healing time to the ulcer area (day/mm^2)	3.75 ± 3.4	5.3 ± 5.1	-	[56]
*Scar area (mm^2)	7.2 ± 15.2	3.8 ± 7.8	Small and translucent with bull's eye appearance	[47, 56, 61]
*Scar to ulcer ratio	0.72 ± 0.52	0.77 ± 1.13	-	[56, 61]
#Surgical therapy	(14-15.6) %	(24.4§ - 30.5) %	-	[57, 70, 71]

CLW = contact lens wear

* non-contact lens-related bacterial keratitis

both contact lens-related and non-contact lens-related bacterial keratitis

†contact lens-related bacterial keratitis

‡ a median score is derived from the proportions of each bacterial type in contact lens-related bacterial keratitis.

§ *Staphylococcus* spp. = *S. aureus, S. epidermidis* and other coagulase-negative *Staphylococci*

Surgical therapy includes lamellar keratectomy, penetrating keratoplasty, enucleation and evisceration.

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894 Table 3. Other CXC chemokines associated with *Pseudomonas* and *Staphylococcus* corneal

895 infection

Author, Date (Reference)	**Model**	***P. aeruginosa* infection**	***S. aureus* infection**	**Remark**
Osthoff et al. 2014 [125]	*in-vitro* infection of HCEC (cadaveric donors)	↑IL-8mRNA at 18 hours PI	-	-
Zhu et al. 2008 [126]	*in-vitro* HCEC challenged with *P. aeruginosa* PAO1	↑IL-8	-	Quorum-sensing signal molecule (OdDHL) altered IL-8 in a dose-dependent manner
Zhang et al. 2005 [34]	*in-vitro* HUCL and primary HCEC challenged with *P. aeruginosa*	↑IL-8mRNA	-	NF-kB facilitates IL-8 expression
Heimer et al. 2010 [131]	*In-vitro* HCEC challenged with *S. aureus*	-	↑CCL20, IL-8, CXCL1, CXCL2, CXCL3 mRNAs	CCL20 was the most abundant chemokine mRNA expressed independent of TLR2 and NOD2
Xue et al. 2001 [129]	*in-vitro* immortalised HCEC challenged with *P. aeruginosa*	↑IL-8 between 8 and 12 hours PI	-	IL-1β mediated the upregulation of IL-8.
Marino et al. 2015 [130]	*ex-vivo* Rabbit model (cornea was excised and challenged with UV-killed bacteria)	-	↑IL-8mRNA	It indicated the immediate innate response specific to *S. aureus* stimulation
Xue et al. 2003 [81]	*in-vivo* BALB/c mice corneal infection (Scratch model)	↑ KC between 16 hours and 3 days PI	-	IL-1β regulated the activity of KC protein
Xue et al. 2002 [113]	*in-vivo* BALB/c mice infection (Scratch model)	↑KC mRNA between 8 and 24 hours PI	-	Cytotoxic and invasive strains caused upregulation of KC.
Cole et al. 2014 [101]	*in-vivo* CXCR2 knockout mice and BALB/c mice infection (Scratch model)	-	↑ KC 24 hours PI	Upregulated KC did not lead to effective neutrophil recruitment.
Cole et al. 2003 [32]	*in-vivo* IL-10 ko mice and B6 mice (WT) infection (Scratch model)	↑KC in 7 days PI	-	The level of KC was correlated with increased angiogenesis
Cole et al. 2000 [135]	*in-vivo* BALB/c mice infection (Scratch model)	↑ KC mRNA between 4 and 24 hours PI	-	KC was located in the epithelium and corresponding to neutrophils in the stroma
Bryant-Hudson et al. 2012 [133]	*in-vitro* B6 (WT) and CXCL1 ko mice infection	↑CXCL1, CXCL2 in WT mice	-	In CXCL KO mice, bacterial counts were elevated between 12 and 24 hours PI.
Xue et al. 2007 [134]	*in-vivo* BALB/c mice infection (Scratch model)	↓CCL2 and CCL3	-	Anti-CCL2 and anti-CCL3 antibodies reduced corneal infection, neutrophil recruitment and the level of IL-1β, MIP-2, KC, VEGF between 1 and 7 days PI.

HCEC = human corneal epithelial cells, HUCL = human telomerase-immortalised HCEC line, HCF = human corneal fibroblast, PI = post-infection, ko = knockout, WT = wild type, IL-8 = interleukin-8, LL37 = Cathelicidin, NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells, CCL2 = C-C chemokine ligand-2, CCL3 = C-C chemokine ligand-3, CCL20 = C-C chemokine ligand-20, CXCL1 = CXC chemokine ligand-1, CXCL2 = CXC chemokine ligand-2, CXCL3 = CXC chemokine ligand-3, IL-1β = interleukin-1β, CXCR2 = C-X-C chemokine receptor-2, TLR2 = Toll-like receptor-2, NOD2 = Nucleotide-binding oligomerization domain-containing protein 2, KC = keratinocyte-derived chemokine (also known as CXCL1), MIP-2 =macrophage inflammatory protein-2, VEGF = vascular endothelial growth factor, OdDHL = n-(3-oxododecanoyl)-l-homoserine lactone, G-CSF = granulocyte colony-stimulating factor

Table 2. Pathology of contact lens-related bacterial corneal infection in non-scratch animal models

Author, Date (Reference)	**Models**	**Experimental conditions**	**Key findings**
Li et al. 2020 [27]	*P. aeruginosa* corneal infection (ATCC 19660) in B6 mice (non-scratch model)	B6 mice were fitted with *P. aeruginosa* colonised soft CL (Hilafilcon B). Additionally, subconjunctival norepinephrine was applied in the case group and PBS in controls. Eyes were evaluated after 48 hours.	• The severity of bacterial keratitis was associated with the level of neutrophil recruitment, and proinflammatory proteins (TNF-α, IL-1β and IL-6) more in norepinephrine treated cases than that of controls. • Extended CLW elevated norepinephrine promoting pathogenesis of CL-induced *P. aeruginosa* keratitis.
Metruccio et al. 2019 [25]	*P. aeruginosa* (PAO1-GFP) corneal infection in B6 mice (non-scratch model)	B6 mice were fitted with SiH CL with and without colonised with *P. aeruginosa* (PAO1-GFP). Contralateral eyes served as controls. Wild B6 mice, along with MyD88 knockout and IL-1R knockout mice, were also examined.	• Microbial keratitis occurred from 24 hours post CL fitting (prevalence = 9%) to 11 days (prevalence = 55%). • CLW with bacterial colonisation increased IL-1R and MyD88 dependent neutrophil recruitment into the corneal stroma after a minimum of five days of continuous CLW. • Additionally, CLW increased dendritic cell recruitment to the central cornea between 24 hours until six days. • CLW without bacterial colonisation remained free of visible pathology.

Wei et al. 2014 [82]	*P. aeruginosa* (invasive strain) corneal infection in rabbits (non-scratch model)	New Zealand white rabbits were fitted with HCL (PMMA or tisilfocon A) colonised with *P. aeruginosa* (strain 6487) for three days.	• Infectious keratitis was more severe and frequent in tisilfocon A (high oxygen transmissible) lens than PMMA lens. • Bacterial adherence to both CLs was comparable. • The severity of infection correlated with neutrophil recruitment.
Szliter et al. 2006 [72]	*P. aeruginosa* (strain 19660) corneal infection in Lewis rats (non-scratch model)	Female Lewis rats were fitted with Lotrafilcon A lenses for 72 hours. An extended CL wear group was challenged with *P. aeruginosa* colonised on the lens surface and served as a case group, whereas PBS was used for controls.	• The level of neutrophils increased in *P. aeruginosa* challenged cornea. • The IL-6 and IL-1β increased at both mRNA level and protein level in the challenged cornea.

PBS = phosphate buffer saline, IL = interleukin, CLPU = contact lens-associated peripheral ulcer, SiH = silicone hydrogel lens, CL = contact lens, CLW = contact lens wear, TNF-α, = tumour necrosis factor, IL-1β = interleukin-1β, IL-6 = interleukin-6, IL-1R = interleukin-1 receptor, MyD88 = (Myeloid differentiation primary response 88), PMMA = polymethylmethacrylate, HCL = hard contact lens.

B6 mice = C57BL/6 strains of susceptible Th1 responding mice, BALB/c mice = strains of susceptible Th2 responding mice, wild type mice= sterile strains of mice, female Lewis rat = susceptible to infection.

P. aeruginosa strain ATCC 19660 = a laboratory strain is known to produce severe keratitis in experimentally infected mice (a cytotoxic strain), *P. aeruginosa* strain PAO1 = a prototypic wild-type strain (poorly virulent laboratory reference) and strain PAO1-GFP = PAO1 transformed with plasmid pSMC2 expressing enhanced GFP.

S. aureus strain 8325-4 = a strain produces both α-toxin and β-toxin and stimulates extracellular release of a proteolytic enzyme and hyaluronidase, *S. aureus* strain DU1090 = an α-toxin deficient of strain 8325-4

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