Review

Epidemiology and Genetic Factors Associated with *Acanthamoeba* Keratitis

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Review

Epidemiology and Genetic Factors Associated with *Acanthamoeba* Keratitis

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Abstract: *Acanthamoeba* keratitis (AK) is a severe, rare protozoal infection of the cornea. *Acanthamoeba* can survive in diverse habitats and at extreme temperatures. AK is mostly seen in contact lens wearers, whose lenses have become contaminated, or a history of water exposure, and in those without contact lens wear, who have experienced recent eye trauma involving contaminated soil or water. Infection usually results in severe eye pain, photophobia, inflammation, and corneal epithelial defects. The pathophysiology of this infection is multifactorial, including the production of cytotoxic proteases by *Acanthamoeba* that degrades the corneal epithelial basement membrane and induce the death of ocular surface cells, resulting in degradation of the collagen-rich corneal stroma. AK can be prevented by avoiding the risk factors, which include avoiding water contact such as swimming or showering in contact lenses, and wearing protective goggles when working on the land. AK is mostly treated with an antimicrobial therapy of biguanides alone or in combination with diaminidines, although the commercial availability of these medicines is variable. Other than anti-amoeba therapies, targeting host immune pathways in *Acanthamoeba* disease may lead to the development of vaccines or antibody therapeutics which could transform the management of AK.

Keywords: acanthamoeba; cornea; keratitis; contact lens; pathophysiology; immunology; microbiology

1. Introduction

Acanthamoeba spp. are common free-living amoebae that are present in a diverse range of habitats, including seawater, swimming pools, tap water, hot springs, soil, dust, and even in the nasal mucosa of asymptomatic individuals [1–7]. Pathogenic strains of *Acanthamoeba* can cause severe, life-threatening infections in immunocompromised individuals [8], and they can also lead to serious blindness arising from *Acanthamoeba* keratitis (AK) [9,10]. AK is a less-known corneal infection first reported in 1974 by Nagington and others [11]. Due to its masquerading symptoms, AK is commonly misdiagnosed as viral or bacterial keratitis, resulting in delayed diagnosis and inappropriate treatment [12,13]. Consequently, AK often results in significant loss of sight even after prolonged treatment.

2. Epidemiology

The main risk factor for AK is contact lens (CL) wear, especially in high-income countries where more than 90% of AK patients have a contact lens history [14]. Other factors such as eye trauma, exposure to contaminated water, or exposure to foreign agents like dirt or algae also increase the risk of AK [15–18]. Studies have also reported increasing non-contact lens-related AK cases, especially in low-middle-income countries where contact lens wear is less prevalent and AK is mostly associated with corneal injury and exposure to contaminated soil or water [19,20]. While AK is usually a unilateral eye infection, it can also infect both eyes [21,22]. The association between the use of contact

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lenses and the high incidence of AK was initially documented in 1984 [23]. A study observed a low global incidence of AK from 1980 to 1990, at approximately 1 to 2 cases per million contact lens wearers [24]. As the number of contact lens wearers increased, the burden of disease similarly rose [25].

In the United States, the incidence of AK is estimated to be one to two new cases per 1 million contact lens wearers annually [26]; approximately 17 % of the U.S. adult population wears contact lenses [27], so with a population of 340 million people, there may be up to 60 cases per year. Epidemiological studies show that the incidence of AK may have increased in recent years, in high-income countries [28–30]. Recent outbreaks have been linked to the use of multi-purpose solutions among soft contact lens users [13,31]. A study conducted in the state of Iowa reported that the annual average of new AK cases per year saw a continuous rise, climbing from 2.9 cases between 2002 and 2009 to 6.5 cases from 2010 to 2017 [17]. Another study at 13 eye centers around the United States found that from 2004 to 2007, a sharp and sudden surge in AK cases was observed. The yearly diagnosis count escalated from 22 cases in 1999 to 43 cases in 2003, eventually reaching 170 cases per year by 2007. This outbreak was linked to the use of the Complete Moisture Plus contact lens disinfection solution [32]. Whilst there was an initial decline in the incidence of AK when this contact lens disinfecting solution was withdrawn from the market, outbreaks have continued, and it is evident that the increase in the number of cases of AK has increased by several orders compared to the period before 2004 [33]. The number of cases seen at Moorfields Eye Hospital in London during the period from 2011 to 2014 (36 to 65 cases per year) was approximately two to three folds higher than during the years 2004 to 2010 (15 to 23 cases per year) [13]. The increase in cases was primarily attributed to using an Oxipol disinfection solution for contact lenses. This disinfecting solution has also now been removed from sale worldwide. Another study conducted in Australia at a prominent referral center in Sydney found that the average annual case count from 2002 to 2016 was 50% higher in the years following 2007 compared to the years preceding it. Notably, the highest numbers of cases were documented in the years 2007 and 2014 [34] .

3. Classification

The classification of *Acanthamoeba* in 1977 was cyst morphology. [35] Using this classification system, at least 31 species of *Acanthamoeba* were identified, which were broadly divided into three major groups. [36] Group 1 has five species characterized by cysts equal to or larger than 18 μm [37]. Group 2, the most prevalent, has 17 species, including several pathogenic species [36,38]. Group 3 has nine species with smaller cysts with less distinct outer walls [37] [39]. However, as the morphology of *Acanthamoeba* cysts can be influenced by the growth medium [40], there was a clear need for a more scientifically robust classification method [41,42]. Therefore, the adoption of molecular techniques has been gaining traction for better identification of the species.

The work on molecular-based classification began in 1996 when *Acanthamoeba* was classified utilizing the whole gene sequence of nuclear small subunit 18S ribosomal RNA (Rns) [43]. This system currently classifies *Acanthamoeba* into 23 genotypes (T1–T23), encompassing all currently known *Acanthamoeba* isolates to date [44–48]. This method was further modified by targeting smaller gene segments, such as 280 base-pair (bp) long highly variable diagnostic fragment 3 (DF3) in the 464 bp long *Acanthamoeba*-specific amplimer (ASA.S1) [49]. This technology has facilitated the subdivision of T2 into two further groups named T2a and T2b [50]. Moreover, T4 has recently been subclassified into eight distinct groups, named T4A, T4B, T4C, T4D, T4E, T4F, T4G/T4Neff, and T4H [38]. Whilst the DF3 fragment has helped in epidemiological and phylogenetic studies, more studies are required to examine its limitations. Therefore, the utilization of full-length sequences of *Acanthamoeba* 18S rRNA is strongly recommended [44,51].

Different genotypes of *Acanthamoeba* are associated with different pathogenicity, disease severity, and clinical presentation. Understanding genotypes may assist in better clinical management of AK [52]. T4 genotype is the most prevalent *Acanthamoeba* genotype in nature [53], and it is identified in the majority of human infections, particularly those associated with AK [54–56]. Most of the *Acanthamoeba* isolates identified from patients with the most severe infections are also

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from the T4 genotype [48], especially T4A sub-genotype followed by the T3 genotype [54]. Other less common genotypes, T2, T5, T6, T8, T9, T10, T11, T12, T13, and T15, have also been recovered from AK patients [57–59]. One study has argued that genotype T5 is not inherently a human pathogen but has recently become more associated with AK due to increased contact lens usage [56].

4. Life Cycle and Morphology

The life cycle of *Acanthamoeba* consists of two stages. They exist as either motile, vegetative trophozoites or dormant, highly resistant cysts [60] [16]. The trophozoite stage dominates when the environmental conditions are favorable for life, such as an abundant supply of water and food, a neutral pH, an optimum temperature (around 30°C), and an osmolarity ranging between 50 and 80 mOsmol [60]. *Acanthamoeba* trophozoites are heterotrophs that primarily consume bacteria, viruses, yeast, algae, or small organic particles through phagocytosis or pinocytosis, forming food vacuoles within the cytoplasm [61,62]. Trophozoites are oval or irregular in shape and are characterized by their typical spine-shaped pseudopods, now called as acanthopodia that extend from the clear ectoplasm [62]. Apart from aiding in movement and feeding, these acanthopodia also help *Acanthamoeba* trophozoites cause corneal infection by adhering to the surface of contact lens [63,64].

Under unfavorable environmental conditions, the trophozoites transform into highly resistant, double-walled, quiescent cysts in a process called encystation [65]. This process involves drastic changes in gene expression, which prepares *Acanthamoeba* to acclimatize to the new environment. Cysts have a rigid, double-layered cell wall which enables *Acanthamoeba* to survive in remarkably harsh environmental conditions such as hyperosmolarity, glucose starvation, desiccation, extreme pH and temperature, the presence of chemicals and toxins, and high doses of radiation [60] [66,67]. Cysts have survived more than 20 years of storage at room temperature with no water or food source and were still able to cause disease when the environmental conditions became favorable [66,68]. The cystic form of *Acanthamoeba* shows minimal metabolic activity, making it highly resistant to anti-amoebal agents [69]. Due to their resistance to contact lens disinfecting solutions and disinfectants, they can contaminate lens storage cases [15]. Cysts are resistant to fungicides, chlorination, and a range of antimicrobials [70,71]. Cysts can remain dormant within corneal tissues for up to 31 months and then produce a recurrence of keratitis [72].

5. Pathogenesis

The pathogenesis of AK occurs in two distinct phases. In the first phase, *Acanthamoeba* attaches to and infiltrates the corneal epithelium, followed by the invasion of the underlying stroma in the secondary phase. In contact lens associated AK, *Acanthamoeba* adheres to the back surface of the contact lens, perhaps from contaminated lens disinfecting solutions, lens storage cases or from low level environmental sources, such as domestic water, and subsequently transfer to the corneal surface. Adhesion is facilitated by acanthopodia in trophozoites [64], but cysts can also adhere to contact lenses [73]. Trophozoites or cysts may be cleared from the ocular surface by tears, the blinking action of the eyes, and the natural immune response of the eyes [74]. However, contact lenses reduce post-lens tear exchange thereby facilitating attachment to the corneal surface [39]. Additionally, contact lens use may induce micro abrasions to the corneal epithelial surface, conceivably making it vulnerable to potential attacks by pathogenic microbes [39].

Acanthamoeba produces adhesive proteins, such as mannose-binding proteins (MBPs) and laminin-binding proteins (LBPs), which bind mannosylated glycoproteins and laminin glycoproteins, respectively on the corneal epithelium [75] [76]. Pathogenic strains of *Acanthamoeba* exhibit high levels of MBPs and LBPs [77,78]. In contrast, non-pathogenic *Acanthamoeba* have fewer acanthopodia per cell (<20 acanthopodia/cell compared to >100 acanthopodia/cell in pathogenic strains) and low levels of binding to MBPs and LBPs, resulting in a reduced binding capacity to adhere to host cells [24]. Initially, the infection is limited to the epithelium, but *Acanthamoeba* can penetrate the epithelial barrier by direct killing and inducing apoptosis of cells. After the breakdown of the epithelial layer, the disease progresses to the next phase, in which trophozoites attach to the underlying collagenous stroma. *Acanthamoeba* can also produce phospholipases that disrupt the host cell plasma membrane.

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This results in the loss of cellular integrity, followed by lysis [79,80]. Neuraminidase can target sialic acid in the corneal epithelium, causing damage to the epithelial cells and facilitating *Acanthamoeba* colonization [81,82]. The combined effect of these factors may contribute to the destruction and dissemination of *Acanthamoeba,* which degrades further into the stromal matrix [83,84].

Acanthamoeba produces three distinct types of proteases: serine proteases, cysteine proteases, and metalloproteases [39]. Serine proteases are the most abundant and can be found in nearly all *Acanthamoeba* species [85,86]. Serine proteases break down collagen type 1, which is present in the corneal stroma and regulates corneal integrity. Additionally, serine proteases attack and degrade secretory immunoglobulin A (IgA), immunoglobulin G (IgG), fibrinogen, fibronectin, laminin, fibrin, hemoglobin, plasminogen, and bovine serum albumin. Cysteine proteases are implicated in the degradation of host cells [87] and can hydrolyze various proteins including immunoglobulin A (IgA), immunoglobulin G (IgG), hemoglobin, albumin, and fibronectin [88,89]. Metalloproteinases also contribute to the initiation of cytotoxic effects on the host cells. These metalloproteinases can degrade collagen, plasminogen, elastin, casein, and gelatin [90,91]. Trophozoites then feed on keratocytes and organic particles within the stroma, resulting in keratocyte depletion, a robust inflammatory response, and ultimately stromal necrosis [21,22].

AK is often accompanied by exquisite pain. The reason behind the intense pain linked to AK is not fully understood. However, the movement of *Acanthamoeba* toward chemotactic signals might lead to their association with nerve cells in the cornea. Clinically the disease is characterized by perineural infiltrates [15], which may indicate neurogenic inflammation. Additionally, enzymes such as MIP133 (mannose-induced protein, a serine protease with high cytolytic activity) could play a role in causing damage to the nerves, ultimately contributing to the pain associated with this infection. Advanced disease stages can potentially kill the corneal nerves by sequential activation of caspase-3 and caspase-10 medicated apoptosis with potential sight-threatening effects [14].

6. Innate and Adaptive host immune responses

When *Acanthamoeba* trophozoites attack the ocular surface, they encounter innate immune cells that promptly defend against the initiation of ocular infection [92]. Studies have highlighted the ability of macrophages to kill *Acanthamoeba,* especially when activated by interferon-γ (IFN-γ) [93,94]. Depleting periocular macrophages through the injection of a subconjunctival macrophage-killing drug, clodronate resulted in a significant worsening of AK in the Chinese hamster model of the disease [92]. Similarly, studies have shown promising evidence regarding the ability of neutrophils to kill *Acanthamoeba* cysts and trophozoites *in-vitro* [95]. In a Chinese hamster model, introducing up to a million *Acanthamoeba* trophozoites into the anterior chamber of the eye resulted in a vigorous neutrophil response that efficiently eradicated the considerable trophozoite inoculum within 15 days following the injection [96]. In a separate animal-based study, the introduction of MIP-2 (an animal homologue of human IL-8), a potent chemotactic attractant for neutrophils, directly into the cornea alleviated AK. Conversely, the injection of anti-MIP-2, inhibited neutrophil migration to the infection site, and led to the worsening of the disease [97].

The role of IL-8 is also important in the pathogenesis of AK. In severe cases of AK, IL-8 is expressed at higher concentrations compared to milder cases [98]. This elevated expression is associated with scleritis. IL-8 activates and attracts neutrophils to the sclera, serving as key inflammatory mediators and contributing to the worsening of AK [98]. Unlike most other inflammatory cytokines, IL-8 remains active at the site of inflammation for up to several weeks, leading to sustained inflammation [99]. Additionally, IL-8 is also a part of the Toll-like receptor 4 cascade, which initiates the cytokine response in AK [99]. The prolonged persistence of IL-8 and other cytokines in the cornea could significantly contribute to ongoing inflammation in AK, which would explain the severe clinical symptoms in affected individuals. Furthermore, IL-8 also promotes angiogenesis, which may contribute to ocular neovascularization, a late complication associated with AK [98].

Serological surveys indicate that 90 to 100% of the asymptomatic population with no history of *Acanthamoeba* infections expresses serum IgG antibodies specific for *Acanthamoeba* [100]. This is not

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unexpected as cysts are present in air and dust and are inhaled daily. These antibodies, in addition to IgA which is present in the highest concentrations in tears [24], are very crucial in preventing *Acanthamoeba* adhesion to the host cell by blocking trophozoite movement and neutralizing cytotoxic effects products produced by the amoeba. IgG may also resist the trophozoite's progress during the stromal invasion [101]. Furthermore, secretory IgG antibodies produced in response to mannosylated glycoproteins of *Acanthamoeba* are thought to activate the complement system by attaching with Fc receptors on the neutrophils. Once activated, these neutrophils attack and kill trophozoites [95,102]. Similarly, most of the asymptomatic population show robust T-cell responses to *Acanthamoeba* exposure [14]. The high occurrence of T and B lymphocyte activation in individuals without a history of *Acanthamoeba* infections emphasizes the ubiquitous nature of environmental exposure to *Acanthamoeba*. However, it is important to note that AK is believed to be caused by the transfer of *Acanthamoeba* from contact lens to the corneal surface. The corneal surface is considered an immune-privileged avascular site, and in this case, A*canthamoeba* may not elicit rapid antibodies or T-cell responses on the ocular surface [103]. Both humoral and delayed-type responses are produced by systemic immunization with *Acanthamoeba* antigens, but these responses cannot provide protection against ocular infection [103].

Since corneal infection caused by *Acanthamoeba* does not produce immunity, persistence of this infection is frequent. This persistence is due to the capacity of *Acanthamoeba* to avoid the immune response to the infection, thus being able to persist in host tissue for extended periods..

7. Genetic Variations associated with the severity of disease

Several host genetic variations are associated with severe inflammatory complications in AK patients. Single nucleotide polymorphisms (SNPs) in the CXCL8 gene, which encodes IL-8, were significantly associated with protection from SICs [99]. Conversely, SNPs in the TLR-4 gene have been associated with increased the risk of SICs, and SNPs in Th-17 related genes, (IL-23R, IL-1β, and IL-22) were also found to be associated with increased incidence of SICs in AK patients [99]. A study of non-amoebic microbial keratitis found associations between genetic variations in interleukins and disease severity. SNPs in the IL-6 gene were associated with more severe clinical outcomes [104]. Moreover, variations in DEFB1 (defensins beta 1) gene expression were associated with increased susceptibility to contact lens related keratitis [105]. SNPs in IL-17F were also possibly associated with more severe outcomes of microbial keratitis [106]. .

8. Immune Evasion

Acanthamoeba has evolved several mechanisms to evade the host's immune system and cause chronic infections. This characteristic makes AK a challenging disease to manage with conventional techniques. Whilst the surface antigens of *Acanthamoeba* can elicit a humoral immune response, leading to the production of secretory IgA and serum IgG antibodies, *Acanthamoeba* trophozoites suppress this immune response by secreting serine proteases, which can cleave IgA and IgG [107,108]. There are lower levels of *Acanthamoeba*-specific IgA and IgG antibodies in AK patients when compared to healthy individuals [100,109]. These decreased levels of antibodies enable *Acanthamoeba* to adhere to the host corneal epithelium and survive in the host for a longer period.

Acanthamoeba antigens can activate T-cells and sensitize delayed-type hypersensitivity responses when injected subcutaneously [110]. However, corneal infections in experimental animals do not induce detectable T-cell-dependent or T-cell-mediated immune responses. The pathological examination of the *Acanthamoeba*-infected cornea revealed the absence of lymphocytes from the AK lesion [111], which suggests that *Acanthamoeba* trophozoites can change their antigenic appearance by masking their surfacing antigens and escape immune recognition from the host immune system [112]. This mechanism may explain why some individuals fail to recover from *Acanthamoeba* infection [113]. As trophozoites are extracellular organisms, their antigens are not presented to the host's major histocompatibility complex (MHC) class I proteins, therefore reducing cytotoxic T-lymphocyte-mediated immunity [114].

The pathogenic strains of *Acanthamoeba* trophozoites express complement regulatory proteins, which can downgrade complement-mediated lysis, making them less susceptible to the antimicrobial effects of the complement [112]. This resistance to the anti-microbial properties of complement may explain why animals immunized against *Acanthamoeba* and expressing complement-fixing antibodies are not protected against AK. A systematic analysis has identified a unique protein secreted by *Acanthamoeba* isolates, M28 aminopeptidase (M28AP), which can degrade crucial human complement proteins, such as C3b and iC3b [115]. This characteristic disables the innate arm of the complement system, enhancing the pathogenicity of *Acanthamoeba*. Pathogenic isolates of *Acanthamoeba* have greater resistance to complement compared to non-pathogenic isolates [116,117]. It is important to note that other pathogenic protozoa, like *Naegleria fowleri* also demonstrate a similar pattern to complement-mediated lysis [118].

A study also observed a strong association between *Acanthamoeba* and the formation of biofilms [115]. Notably, biofilms play a crucial role in facilitating infection by providing a protective environment for *Acanthamoeba*, helping in immune evasion. These biofilms are believed to enhance *Acanthamoeba*'s invasiveness and are also thought to serve as a source of nourishment for the organism [81,119].

Acanthamoeba trophozoites undergo encystation within human tissue as a strategy to escape the host immunologic response. This characteristic enables *Acanthamoeba* to resist immune attacks and survive in a hostile host environment. Studies have shown that cysts are less chemoattractant for macrophages and lymphocytes [120]. Despite successful treatment, cysts can persist in the corneal stroma for several years, where they are less immunogenic and fewer in number than trophozoites [114]. Additionally, the eye's immunologically privileged nature provides further protection to these residing cysts, preventing recognition and elimination by the adaptive immune system [121] [109]. These cysts are a crucial source for the recurrence of corneal infections [114].

9. Diagnosis

Acanthamoeba is a rare cause of keratitis, which is why it is often overlooked as a differential diagnosis by clinicians. Early diagnosis of AK is essential to avoid serious damage to the cornea and vision. It is important to have a clinical suspicion of AK in patients with associated risk factors. The different diagnostic techniques have their strengths and limitations. Therefore, multiple diagnostic approaches are used for the diagnosis of AK. In clinical settings, culture is most commonly used along with in vivo confocal microscopy (IVCM) and polymerase chain reaction (PCR).

The culture of *Acanthamoeba* is considered the gold standard and plays an important role in diagnosing suspected cases of AK. It is usually performed with corneal scrapings and gives 100% specificity. [122] However, the sensitivity of the culture is reported to be below 67% [123] [124] [125], and is influenced by the culture method used [126]. The technique for obtaining the corneal specimen can also impact the diagnostic sensitivity. Corneal biopsy has the highest sensitivity, followed by spatula methods, while specimens obtained by a cotton swab have the lowest sensitivity [127]. The sensitivity of the culture is improved when combined with a direct smear stained with stains like Calcofluor white, which helps identify *Acanthamoeba* cysts [128]. It is important to note that amoebic cultures require at least 1-2 weeks for a positive result, delaying diagnosis and treatment, which may contribute to the worsening of the disease.

PCR of corneal scrapings are becoming increasingly popular for diagnosing AK, providing faster results with a more accessible diagnostic modality than culture. PCR offers high specificity (100%), and the sensitivity of a single PCR assay for AK is reported to be approximately 70%, which is higher than some culture-based methods, with the potential to increase up to 93% or more when multiple gene assays are used [124] [125]. However, the sensitivity of PCR is reduced in the initial phase of infection, particularly with small specimen sizes and in cases undergoing anti-Acanthamoebal treatment [129]. The limitation of PCR is its inability to distinguish between dead and living organisms. Recently, real-time PCR has become a standard diagnostic procedure mainly because of the rapid delivery of results, which is advantageous. PCR also allows the genotyping of *Acanthamoeba*, which may help in disease management and future epidemiological studies.

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IVCM is a non-invasive diagnostic tool recently employed for AK diagnosis. In comparison to corneal culture and PCR, IVCM is not widely available in healthcare facilities due to its expensive cost. It is operator dependent but considered the primary method for diagnosing AK where it is available. IVCM has a very high specificity (100%) and a high sensitivity, ranging from 85–100% [130] [131]. The test also provides rapid results, especially when compared to other diagnostic tools. This technique can detect *Acanthamoeba* cysts only, which makes it limited to detecting AK at later stages of the disease [132]. Moreover, stromal inflammation and edema can cause masking of *Acanthamoeba cysts,* resulting in false negative results. Apart from diagnosis, IVCM is also used to assess treatment outcomes and examine for residual disease [133].

10. Prevention and ways to reduce poor outcomes of AK

Increased awareness regarding the associated risks of poor contact lens hygiene practices is important. Key practices include avoiding contact between tap water and contact lenses by not handling lenses with wet hands, and not wearing contact lenses when showering or swimming. Hand hygiene is important, so washing hands with soap and water and drying them thoroughly before handling contact lenses may help to reduce the risk of corneal infection. The use of self-made saline solutions and expired disinfectants is highly discouraged. It is necessary to use only approved disinfecting solutions for CLs cleaning and storage. Failure to clean or replace contact lens cases is associated with a higher risk of severe infection.

Poor outcomes of AK can be improved using a multi-dimensional approach. The development of *Acanthamoeba*-specific IgA antibodies in tears, which are capable of blocking trophozoite adherence to mannosylated proteins on the corneal epithelium, can be effective in preventing infection. Moreover, identifying and targeting various proteases secreted by *Acanthamoeba*, such as the serine protease MIP-133 known for degrading corneal cells, can be crucial in disease management. In animal models, oral immunization with MIP-133 induced the production of IgA in tears, which reduced AK severity [134]. These findings could be potentially translated to humans. Furthermore, topical application of anti-MIP-133 antibodies to the corneas of AK patients may help neutralize the proteases, potentially improving clinical symptoms. Further research is necessary to explore the potential role of protease inhibitors as treatments.

Acanthamoeba secretes another protein, M28AP (M28 aminopeptidase), which degrades human complement proteins such as C3b and iC3b and disables the host's immune system. Targeting this protein may also be an avenue for therapy. Moreover, *Acanthamoeba* trophozoites transform into cysts in the cornea, which enhances its pathogenicity and results in the recurrence of infection after treatment. Developing treatments that can target distinct morphological structures of cysts may offer further clinical management options.

Understanding and targeting the pathways through which IL-8 contributes to the inflammatory response in AK is crucial for developing targeted management strategies and improving therapeutic outcomes for affected individuals. The identification of variations in IL-8 levels among patients who develop neovascularization may provide insights into anticipating and managing AK complications more successfully. Additionally, genetic screening for specific SNPs associated with SICs in AK patients could help predict the risk of disease severity, allowing for more personalized and targeted management of the disease.

11. Conclusion

AK presents a rare yet serious corneal infection, posing significant challenges in both diagnosis and treatment. The infection is caused by a ubiquitous protozoon *Acanthamoeba* and particularly affects vulnerable groups, notably contact lens wearers and those who have recent eye trauma. AK is often misdiagnosed, resulting in delayed intervention and the risk for vision loss. Understanding the immunopathology of *Acanthamoeba* is important for effective disease management. The complex interaction between the microbe and host immune system contributes to the severity and complexity of AK. *Acanthamoeba* uses different mechanisms to evade the host's immune system and persists in host tissue, making it challenging to manage with conventional techniques. Genetic variations in host

genes further contribute to the severity of the disease. The host immune response to the infection is crucial for disease eradication but it simultaneously poses risks such as scarring and vision loss due to increased corneal inflammation. Exploring avenues like genetic screening for host susceptibility, antibody therapeutics, and specific protein targets of *Acanthamoeba* may lead to improved treatment outcomes.

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