Atypical Mycobacterial Keratitis: A Negligent and Emerging Thread for Blindness

Thet Tun Aung1, Roger W Beuerman1,2,3*
1Singapore Eye Research Institute, Singapore 169856
2SRP Neuroscience and Behavioral Disorders, Duke-NUS, Singapore 169857
3Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228

ABSTRACT

Atypical mycobacteria are widely spread in the environment; they are now known to be a cause of a variety of infections including corneal infections especially after refractive surgery. The diagnosis and clinical management are often unsatisfactory due to misdiagnosis and requirement of prolonged combination of antibiotics. Evolving drug resistance is known to be a unique feature in managing atypical mycobacterial keratitis due to the propensity for the development of biofilms. In this article, we provide an etiology of atypical mycobacterial keratitis, mycobacterial biofilm pathogenesis, and the importance of mycobacterial biofilm matrix component (extracellular DNA) in maintaining mycobacterial biofilm matrix maintenance. Current treatment options for atypical mycobacterial keratitis are summarized and suggestions are made for the new treatment strategies targeting on the mycobacterial biofilm pathogenesis pathway.

Etiology of Atypical Mycobacterial Keratitis

Bacterial keratitis is an important cause of corneal opacification and blindness across the world, but particularly so in South East Asia1. Several types of pathogenic organisms can lead to keratitis, inflammation of the cornea of the eye with potential corneal perforation, sight-impairing fibrosis and loss of vision1. Causal organisms for keratitis include bacteria, fungus, virus, protozoa and parasites and the risk factors include ocular trauma and surgery, contact lens wear, pre-existing ocular morbidity, dry eye, lid deformity, systemic immunosuppression and prolong usage of topical steroids2-4. In recent years, there has been an emerging trend of atypical mycobacterial (AMB) corneal infections. The rising use of biomaterials, increasing number of refractive surgery procedures [LASIK (laser-assisted in situ keratomileusis), endothelial keratoplasty] and the expanded use of fluoroquinolone prophylaxis result in the rising AMB keratitis cases5. AMB are often referred to as nontuberculous mycobacteria, and these aerobic, non-motile, non-spore forming bacilli have been documented in many reports of ophthalmic infections such as keratitis, canaliculitis, uveitis, dacryocystitis, orbital cellulitis, and endophthalmitis6-8. AMB keratitis is a leading cause of post-LASIK infections as noted by the American Society of Cataract and Refractive survey (ASCRS) Cornea Clinical Committee9. Among the different ocular morbidities caused...
by atypical mycobacteria, infectious keratitis is seen most commonly. AMB keratitis accounts for 3 percent of total different bacterial keratitis cases in the United States in 2013. There were reports suggesting that Mycobacterium fortuitum (M. fortuitum) and Mycobacterium chelonae (M. chelonae) are the most prevalent causal strains for AMB keratitis.

AMB infections are endemic in nature, widely distributed and varied in nature depending upon the environmental factors such as geographical status, climate and soil. Mode of transmission of AMB infections is poorly understood, but they are widespread throughout the world and accounting for 10 percent of total mycobacterial infections other than Mycobacterium tuberculosis (M. tuberculosis) in the United States. One of the unusual characteristics of AMB is the lack of evidence of person-to-person transmission like M. tuberculosis. The latent forms of AMB may be present in a normal person. However, AMB may turn into an active infection when a person loses their natural immunological capacity due to other predisposing factors. It has been postulated that AMBs are opportunistic infections resulting lesions in the lowered resistance regions. Children and older adults with chronic diseases are more susceptible to AMB infections. There were reports suggesting that a history of corneal insults with a latent period was the main barrier leading to the poor management of AMB keratitis. The portal of entry for these AMB infections is direct inoculation or entry into the skin or cornea. In keratitis cases, it is possible that AMB infections are caused by either direct contact with biomaterial or dust containing airborne mycobacteria.

**Atypical Mycobacterial Biofilms**

Bacterial pathogens are able to form surface-attached complex multicellular biofilm communities and according to National Institute of Health, a biofilm phase is present in 80% of all bacterial infections, including conditions such as gingivitis, infectious kidney stones, bacterial endocarditis, and inner ear infections, along with many hospital-acquired infections from catheters and ports. Bacterial cells within biofilms are surrounded by extracellular polymeric substances (EPS) consisting of extracellular proteins, extracellular polysaccharides and extracellular DNA (eDNA), and have a distinct physiology compared to their free-living counterparts. Formation of biofilms noticeably increases the bacterial cells’ tolerance toward antimicrobials as well as to attack by the host immune system. Biofilm formation is responsible for a large percentage of treatment failures for infections and causing a huge economic burden for our health care system. Increasing numbers of reports suggest that different infections including ocular infections involve biofilm formation as a major therapeutic burden. Recent reports suggest that Pseudomonas aeruginosa (P. aeruginosa) biofilms are found on the mucosa of the lungs in cystic fibrosis and the cornea, both are epithelial surfaces. The classical stages of biofilm formation in experimental infections (P. aeruginosa and atypical mycobacteria) of mouse cornea have provided evidence of biofilm formation using EPS, bio reporter strains and eDNA as biofilm markers. Therefore, we suggest that bacterial biofilms play a critical role in AMB keratitis in several ways:

1. Mycobacterial biofilms serve as a reservoir that can release bacteria leading to the secondary infection.
2. Mycobacterial biofilms are resistant to conventional therapeutic options, making it difficult to eliminate the bacteria.
3. Lateral gene transfer can occur in biofilms encouraging resistance.
4. Failure of conventional antibiotics and host immune responses to eliminate the infection lead to chronic infection, which in turn produces a chronic inflammatory response and leading to tissue damage apart from damage by bacteria directly.

**Importance of Extracellular DNA in Atypical Mycobacterial Biofilm**

EPS has been recognized as a primary component of biofilms. However, eDNA has now been found to be equally important. The controversial issue of the source of eDNA in bacterial biofilms is an ongoing. Cell lysis is the major mechanism providing eDNA in P. aeruginosa, Staphylococcus spp and Enterococcus spp. Autolysins proteins, pyocyanin leading to H₂O₂ production are major factors in the cell lysis. However, there has been a growing interest of research regarding active eDNA export as an alternative source of eDNA. One report suggested that there was no evidence of cell lysis in Enterococcus faecalis early biofilms with an abundance of eDNA. Secreted membrane vesicles were reported to be responsible for active export of eDNA in biofilms. It was found that active export of eDNA in atypical mycobacteria was positively targeted by bicarbonate. The functional importance of eDNA in the biofilm has been shown and there have been several reports showing the presence of eDNA in biofilms of different bacteria species. eDNA plays a major and universal role in the regulation of the biofilm network and integrity in most bacterial species. Horizontal gene transfer between bacteria is another factor leading...
to antibiotic resistance in the clinic which was first investigated in 192844. Moreover, it has been discovered that the eDNA pool in a bacterial biofilm provides a rich medium for genetic transformation naturally, other than bacteriophage induced gene transfer and mobile genetic elements45. Studies suggest that eDNA can increase gene expressions which could lead to antimicrobial resistance46. There were reports shown that eDNA is a major component of the mycobacterial biofilm matrix and DNase 1 aids the efficacy of antibiotics in treating infections in vitro48, 49. Moreover, an addition of exogenous eDNA enhances the biofilm formation in vitro, suggesting again that eDNA plays a major and integral part of mycobacterial biofilm matrix maintenance and regulation53.

Clinical Features and Diagnosis

Symptoms of AMB keratitis such as decreased vision, a variable degree of pain and photophobia are usually present in gradually increasing patterns, probably due to the spread of the infection within the cornea5. The clinical features are less severe in post-LASIK AMB keratitis than that of cases caused by trauma19. The variable clinical presentations of AMB keratitis cases, multifocal or a single lesion surrounded by multiple white satellite lesions were reported7, 20. Most AMB keratitis cases showed an intact corneal epithelium at their initial presentations50. Hypopyon is present in poorly managed AMB keratitis cases and the anterior chamber usually appears to be normal5. An unique clinical sign, ‘cracked windshield’ appearance, due to radiating corneal infiltrates by AMB infections has been noted (Figure-1)51. Intrapstromal opacity, infectious crystalline keratopathy and minimal inflammation have been reported in some of the cases of AMB keratitis52. The clinical diagnosis of AMB keratitis is often delayed due to the slow growing nature of the organisms5. Corneal scraping from the lesions is required to identify the AMB and the gold standard of the laboratory detection is culturing on specific solid agar media such as Lowenstein-Jensen medium, MacConkey agar, Middlebrook 7H10 and 7H11 media19. Molecular PCR-based technology has been developed to detect AMB infections, which are relatively faster and more specific compared to traditional microbiological methods53. Primers targeting the heat shock protein 65 (hsp65) have been utilized to detect M. fortuitum in postoperative endophthalmitis patients54.

Therapeutic Options currently available for Atypical Mycobacterial Keratitis

The rarity of the disease in clinical scenarios can result in complications in dealing with AMB infectious keratitis. In one report, 4 months was required for the diagnosis of a mycobacterial infection in South Florida55. Clinical management of AMB keratitis is not efficient because of the delayed diagnosis and prolonged drug sensitivity tests due to their long incubation time to grow the bacteria in the lab. Moreover, there are several reports showing variations in MIC susceptibilities of M. fortuitum and M. chelonae against different antimicrobials56. According to the literature reviewed, antibiotics showed a large range of antimicrobial sensitivities, for the effective treatment of M. fortuitum and M. chelonae56. Amikacin has been the gold standard treatment for AMB keratitis. However, it has been shown that amikacin is ineffective in the management of AMB keratitis and unable to act efficiently in the intact corneal epithelium57, 58. Recent reports showed that fluoroquinolones are a better choice for treatment and have an excellent antimicrobial activity against AMB keratitis.59, 60. However, there are reports of resistance evolving for AMB against the fluoroquinolones59, 60. Combination of clarithromycin and amikacin haven been used to treat AMB infections61, 62. On the other hand, AMB infections in and around the eye are clinically recalcitrant to treatment, recur after cessation of therapy, and require long-term therapy to eradicate the infection. Currently there is a lack of antibiotics and settled treatment strategy that can efficiently eradicate AMB infections. The above-mentioned factors and a low index of clinical suspicion result in the inefficient management of AMB keratitis cases.

Conclusion

Although AMB keratitis cases are increasing, the understanding of the pathogenesis of AMB keratitis and effective treatment options are still limited. Studies have shown that M. fortuitum and M. chelonae readily form drug-resistant biofilms, irregular finger-like projections and microcolonies (Figure-2), suggesting a probable cause of poor treatment compliance to conventional antibiotics48. Studies have concluded that there is a significant eDNA release with M. fortuitum and chelonae biofilms, which protect the organisms from the antibiotics: however, adding DNase can boost the efficacy of available antibiotics33, 48, 49. There was a pioneer report to show that AMB keratitis is a biofilm mode of growth in an experimental mouse.

keratitis model and suggested that poor clinical outcomes of AMB infections might be due to its presence of biofilm matrix causing a barrier for antibiotic penetration and resistance. The clinical management of AMB keratitis was often unsatisfactory due to its potential to acquire resistance because of its single agent therapy and unsettled treatment guideline. Therefore, clinicians prescribe drug combinations in order to reduce drug resistance problems and treat AMB keratitis effectively. Since there was a report suggesting a strong synergism between DNase, aminoglycosides, and fluoroquinolones against AMB infections, a consideration for a new standard treatment might be using these three different drugs in combination in any suspicious AMB infection cases. Future studies need to focus these three drugs in an optimal combination against different clinical isolates with validation in clinical trials.

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Conflict of Interest
Nil

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