Mycobacterium avium Complex

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Slow-growing mycobacteria, Mycobacterium avium, and rapid-growing mycobacteria, Mycobacterium abscessus, are important emerging human pathogens causing disease in vulnerable populations such as severely immunocompromised patients, patients with chronic lung diseases, and others with underlying health conditions. Their incidence is increasing worldwide in industrialized countries and they cause substantial yet underacknowledged burden of disease. An overview of the general characteristics, vulnerable populations most at risk, pathogenesis, treatment, and prevention for infections caused by Mycobacterium avium, in the context of MAC, and M. abscessus are presented in this review.

Keywords: atypical mycobacteria ; Mycobacterium avium ; Mycobacterium abscessus

1.Introduction

The most common NTM species causing human disease are the slow-growing mycobacteria *Mycobacterium avium* in the *Mycobacterium avium* complex (MAC), the rapid-growing *M. abscessus,* and *M. kansasii*.

2. General Characteristics

Mycobacterium avium complex (MAC) infections are caused by a group of mycobacteria consisting of *M. avium*, *M. intracellulare*, and the newly described mycobacterium species, *Mycobacterium chimaera*, which are all acid-fast slow-growing mycobacteria, classified as non-chromogens in group III of the Runyon classification of NTM. MAC initially included only *M. avium* and *M. intracellulare*, but with new genetic sequencing technology, phenotypic and genotypic tests have identified additional species. To date, MAC contains nine species of slow-growing mycobacteria: *M. avium, M. intracellulare, M. chimaera, M. colombiense, M. marseillense, M. timonense, M. boucherdurhonense, M. vulneris, M. arosiense*, and a small group of unclassified, “MAC others”[1]. Recently, *M. chimaera* was implicated in worldwide outbreaks in five countries including the USA, Europe, and Australia, due to contaminated heater–cooler unit water tanks in patients that underwent open-heart surgery, such as coronary artery bypass grafting[2]. Investigations found the culprit was contamination from the Stöckert 3T heating and cooling unit manufactured by LivaNova PLC in London[3]. The sequence of the 16-23S internal spacer (ITS) region of *M. chimera* corresponds to the sequevar MAC-A compared to the *M. intracellulare* sequevar MIN-A (DSMZ 43223) by 20 nucleotide (nt) mismatches. Additionally, the 16S rRNA gene sequence between the two species only has a 1 nt mismatch. Since the sequence of 16S rDNA is considered the approved standard for identifying NTM species, *M. chimaera* is usually misreported as *M. intracellulare*[4][5]. Traditionally, standard molecular genetic tools in clinical microbiology laboratories did not differentiate MAC members and only gave a rough classification of *M. avium*, *M. intracelllulare*, or MAC as a whole since treatment is the same for all MAC species. Detailed genotyping of MAC species was limited to specialized research laboratories that can perform PCR of several genes such as rpo or hsp65 and ITS[6]. However, due to these recent outbreaks associated with cardiac surgery, clinical laboratories are now mandated to differentiate *M. chimaera* from other MAC species[2].

The most clinically significant organism for human disease within MAC is *Mycobacterium avium*, with four distinct subspecies. This group of bacteria ranges from environmental bacteria that can cause opportunistic infections in immunosuppressed individuals to pathogens for birds and other animals. The characteristics of the four distinct species of *M. avium* are discussed. *M. avium* subsp. *Hominissus* (MAH) is an opportunistic environmental pathogen for humans, swine, and other animals that are found in soil and water. Drinking water and tap aerosols are considered the primary sources for infection in humans. MAH is clinically the most relevant organism within MAC for humans and a major pathogen for individuals with deficient T cell immunity. Before the development of antiviral therapy, in the early years of the Acquired Immunodeficiency Syndrome (AIDS) epidemic, 20–50% of severely immunocompromised AIDS patients had disseminated infections with MAC organisms, now identified as MAH infections[7]. MAH is still a threat to newly diagnosed HIV patients and individuals with no access to antiviral therapy or for those that antiviral therapy is ineffective[8]. In

immunocompromised individuals, MAH can cause pulmonary infections, cervical infections, mainly in children, and soft tissue infections. *M. avium* subsp. *Paratuberculosis* (MAP) is a widely known pathogen that causes paratuberculosis, also known as John's disease, primarily affecting ruminants, causing chronic progressive infection in the small intestine. Non-ruminants, and non-human primates can be infected with MAP as well. MAP has a worldwide distribution with transmission mainly by the fecal–oral route via pasture, milk, and water contaminated with feces[9][10]. In humans, MAP has been proposed to be linked to Crohn's disease, due to a meta-analysis demonstrating their association, however, the association remains controversial and inconclusive[11]. *M. avium* subsp. *Avium* (MAA) mainly causes a tuberculosis-like disease in birds, the main reservoir of MAA. Avian TB is a chronic wasting disease, showing little signs of infection. A variety of mammals, but mostly pigs and cattle, can become infected with MAA[12]. Transmission is mainly from birds to susceptible animals via ingestion or through the environment with fecal contamination[13]. *M. avium* subsp. *Silvaticum* (MAS) is taxonomically similar to MAA and is a pathogen to the wood pigeon, causing TB-like disease. Some experimental studies also show that, in mammalian species, MAS can cause chronic enteritis[14] [19].

3. Vulnerable Populations to Mycobacterium avium Complex (MAC) Infections

The pathogenesis of *M. avium* depends on its ability to colonize intestinal or respiratory mucosa, evade protective barriers, and resist intracellular killing in macrophages. The disease normally does not develop in most people; however, immunocompromised patients or patients with pre-existing conditions are at higher risk of MAC infections. There are three types of MAC infections that can occur: (1) pulmonary MAC infections, (2) disseminated MAC infections, and (3) MAC-associated lymphadenitis[15].

Pulmonary MAC infections affecting lungs are the most common type. These infections mainly affect patients with chronic lung diseases, such as chronic obstructive pulmonary disease (COPD), chronic bronchitis, bronchiectasis, cystic fibrosis, and lung cancer. Pulmonary infections are likely acquired through inhalation of aerosols from the natural or institutional water systems. The fibrocavitary disease form of MAC is common in older male smokers with chronic pulmonary symptoms. The nodular/bronchiectatic disease form is most common in nonsmoking women over the age of 50 with chronic lung disease[15]. Another predisposition for the nodular/bronchiectatic disease form is particular body morphotypes with similar clinical characteristics including scoliosis, pectus excavatum, mitral valve prolapse, and joint hypermobility[16]. These pulmonary infections are characterized as a localized infection; however, it can spread to submucosal tissue, enter the bloodstream, and infect other organs and tissues. This results in disseminated MAC infections and usually affects patients with HIV/AIDS.

Disseminated MAC (DMAC) infections usually occur in patients in advanced stages of HIV/AIDS when blood CD4+ T cell counts are lower than 50/mm^3, unlike *M. tuberculosis* that can infect patients at any stage of AIDS[17]. MAC is most likely acquired by the gastrointestinal tract in HIV-infected patients[18]. In the developed world, DMAC is the most frequent opportunistic bacterial infection in HIV patients with an annual occurrence of 10–20% in those who have AIDS[19]. Before the availability of antiretroviral therapy (ART), *M. avium* was the etiologic agent in >95% of individuals living with HIV with advanced immunosuppression. In recent years, with the availability of antiretroviral therapy (ART), the rate of DMAC has fallen substantially[8]. However, adults with HIV and advanced immunosuppression who are not receiving or unable to tolerate ART are still at risk of disseminated MAC. Without antiretroviral therapy or prophylactic antibiotics, the incidence of DMAC among HIV-infected patients with CD4 counts less than 100/mm^3 is approximately 20%[19].

MAC can also cause lymphadenitis, mainly cervical lymphadenitis in immunocompromised children under five years old. However, one study indicated that it primarily affects healthy children under 15 years old with normal immune systems, and is an underrecognized cause of lymphadenitis in young children[20][21]. There are also rare Mendelian genetic susceptibilities to mycobacterial disease related to IFN-γ production deficiencies or receptor abnormalities[22][23]. IFN-γ or IL-12 receptor deficiencies cause approximately 80% of the genetically diagnosed cases. These rare monogenic disorders resulting in susceptibility to NTM infections are grouped together as Mendelian Susceptibility to Mycobacteria Disease (MSMD) conditions. Most Mendelian susceptibilities for NTM infections are mutations in genes encoding cytokines, receptors and downstream signaling-transducing proteins of the 1L-12/IFN-γ axis. Mutations in STAT1, a transcription factor also results in an impaired response to type I (IFN-α/β), type II (IFN-γ) or type III (IFNλ-1/2/3) interferons[24]. Mutations in the interferon regulatory factor (IRF)-8 also undermine production of IL-12 in response to IFN-γ[25]. Mutations in interferon-stimulated gene (ISG)-15, whose product is involved in IFN-γ production by T and natural killer (NK) cells, likewise increase susceptibility to NTM infections[26]. The autosomal dominant deficiency of the transcription factor GATA-2 also carries a significant risk to NTM infections; this contributes to a deficiency of mononuclear phagocytes[27][28]. Auto-antibodies to IFN-γ, use of TNF-α inhibitors, mutations in RAR-related Orphan Receptor (RORC), and CD4+ T cell

deficiency are also factors contributing to host susceptibility[15][29]. Phagocyte defects, such as chronic granulomatous disease (CGD), which impair the respiratory burst due to the lack of a functional NADPH oxidase, have also been shown to produce systemic complications in patients receiving the bacillus Calmette–Guerin (BCG) vaccine[30].

Immunosuppressive therapies taken by transplant recipients or patients with immune-mediated inflammatory diseases also lead to a higher risk of NTM infections. The use of corticosteroids, TNF-? treatment, or TNF-α inhibitors significantly increase the risk of developing pulmonary or disseminated NTM infections. One study found that immune-mediated inflammatory disease patients receiving anti-TNF-α treatment had elevated rates of mycobacterial disease and that NTM disease was associated with rheumatoid arthritis (RA)[31]. For organ transplant recipients taking a variety of immunosuppressive mediations such as calcineurin inhibitors (tacrolimus and cyclosporine), mammalian target of rapamycin (mTOR) inhibitors (sirolimus), and prednisone, in one case study, these were shown to be one of the most important risk factors of NTM infection, accounting for 55.9% of cases (19/34)[32].

4. Pathogenesis of Mycobacterium Avium/MAC

4.1. Invasion and Adherence of Mucosal surfaces

These mycobacteria are acquired through the respiratory or the intestinal route, able to invade mucosal epithelial cells and translocate across the intestinal and respiratory mucosa to then infect phagocytic cells[33]. Studies have shown that *M*. *avium* can resist the acidic pH conditions of the stomach and were able to gain access to the intestinal lumen. *M. avium* administered orally are isolated from the jejunum, ascending colon, and the majority in the terminal ileum[34]. Other studies also show *M. avium* preferentially entering the terminal ileum via enterocytes of both Peyer's patches and outside the Peyer's region[33].

Another mechanism associated with *M. avium*'s ability to evade epithelial cells is their interaction with fibronectin-attachment protein (FAP), allowing them to utilize fibronectin like a "bridge" to attach to integrin receptors found on the cell membrane of mucosal cells[35]. Middleton et al. found that MAC can adhere to the extracellular matrix (ECM) in areas of epithelial damage via fibronectin attachment protein (FAP) and to the mucus via another adhesin[36]. Hemagglutinin binding protein has also been linked to the *M. avium* invasion of epithelial cells, although direct evidence is lacking[37].

Once attached to the mucosal cells, *M. avium* triggers uptake on the apical surface, associated with a cytoskeleton reorganization and the phosphorylation of proteins[38]. The precise receptors utilized by the bacterium to infiltrate the mucosal cell are unknown. The entry of *M. avium* is not associated with an inflammatory response[39]. However, there is an association with the suppression of chemokines, interleukin-8 (IL-8), and RANTES in the cells of the epithelium, all of which are mechanisms to reduce the host response to the infection[40]. Mechanisms for the bacterium to exit the polarized intestinal cells are not known at this time, but it has been recognized that the bacterium leaves the mucosal epithelial cell with a phenotype that is significantly more effective with for the invasion of macrophages[41]. *M. avium* may also bypass the mucosa, and then locate to lymph nodes of the mesentery, where it survives until the immune system is compromised, such as with AIDS patients[35].

The pathway for infection of the respiratory tract is less well known than the intestinal route, but initiation is most likely through inhalation of aerosols of the bacteria. *M. avium* respiratory infections usually only affect patients with chronic lung disease, and their establishment of the infection could be due to the development of biofilms, which *M. avium* is known to establish in municipal water systems[42]. Once *M. avium* contacts the alveolar space, it was found they are capable of invading type II alveolar epithelial cells and replicating within these cells[43].

4.2. Interaction with Phagocytes

The primary host cells for *M. avium* and most mycobacteria are in mononuclear phagocytes such as monocytes and macrophages. The bacterium can gain access into monocytes and macrophages by several different receptors: complement receptors, mannose receptors, type A scavenger receptors, and in hosts with mycobacteria-specific antibodies, Fc-gamma receptors[44]. The use of these receptor types is most likely redundant since mice lacking complement receptor CR3 and CR4 were still able to establish an infection indistinguishable from immunocompetent control mice[45]. Once bound to the cell, the mycobacterium is taken up in primary phagosomes that fuse with vacuoles inside the macrophages' cytoplasm. An infection occurs when it survives and proliferates within vacuoles as an intracellular pathogen. Its ability to survive is through immune evasion mechanisms such as the inhibition of phagosome–lysosome fusion, the inhibition of the acidification of the phagocytic vacuole, the disruption of the actin microfilament cytoskeleton, the induction of NTM-related genes to enhance replication, and a shift in metabolism to a more anaerobic intracellular environment[46][47][48][49][50].

4.3. Host Defense and Immune Response

The host defense against *M. avium* involves both the nonspecific (innate) and specific (adaptive) immune system working together and secreting cytokines in response to *M. avium*. The innate immune response contains natural killer (NK) cells, which are critical components of this response, and have been confirmed to play a part in the nonspecific immune response against *M. avium* in mice and human systems[51][52]. Mice with dysfunctional natural killer cells were found to be more prone to an *M. avium* infection[53]. The host's specific immune response to *M. avium* has been shown to be CD4+ T lymphocyte-dependent and based on the generation of IFN-. CD8+ T cells roles are not well defined, but studies have shown that CD4+, but not CD8 T cells are required for adaptive immunity against *M. avium*[54][55].

Macrophages are the central players in the defense against *M. avium*. Macrophages stimulated with cytokines TNF-?, IFN-?, and GM-CSF can control infection[56] and were confirmed in experimental miceRole of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of Mycobacterium avium infection[54]. IL-12 is also a key cytokine in the host defense against mycobacteria. Infected macrophages respond with the production of IL-12, activating NK cells, and T lymphocytes to proliferate and secrete cytokines[57]. NK cells secrete TNF-?, IFN-?, and GM-CSF in response to *M. avium*[58]. When stimulated by NK cell products, infected macrophages were able to control the intracellular infection[59]. Although IL-12 is initially secreted from infected macrophages, there is progressive suppression of IL-12 and an inverse relationship with the period of infection[57]. The exact mechanisms of macrophages killing *M. avium* are less known, but preliminary research suggests that the activation of macrophages gives rise to changes in the vacuoles of the intracellular environment[60].

5. Treatment of Mycobacterium avium/MAC Infections

The *Mycobacterium avium* complex (MAC) consists of most of the common pulmonary NTM pathogens that are heavily linked to the causes of human disease in almost all regions of the world[61]. As previously mentioned, MAC infections consist of (1) pulmonary MAC infections, (2) disseminated MAC infections, and (3) MAC-associated lymphadenitis. According to the American Thoracic Society, Pulmonary NTM infections are categorized even further based on radiologic criteria[62].

5.1. Pulmonary MAC Infections

The three categories of pulmonary MAC infections are as follows: (1) noncavitary nodular bronchiectatic disease, patients who have bronchiectasis clusters with less than 5-mm nodules; (2) cavitary nodular bronchiectatic disease, patients who also present with lung cavities in addition to bronchiectasis; and (3) fibrocavitary disease, patients with cavities, fibrosis, and/or pleural involvement over adjacent areas.

The treatment of pulmonary MAC infections consists of antimicrobial therapy, particularly macrolides, followed by close follow-up monitoring for up to a year. For patients with noncavitary or mild/moderate bronchiectatic disease, the current guidelines recommend a three-drug, macrolide-based regimen (including a macrolide and ethambutol) for macrolide-susceptible MAC pulmonary disease.

For example, a three-drug regimen of azithromycin (500 mg 3×/week), rifampin (600 mg 3×/week), and ethambutol (25 mg/kg 3×/week) can be used (see Table 2). An azithromycin-based regimen is recommended over clarithromycin-based regimens due to its better tolerance, less drug interactions, lower pill burden, and equal efficacy[63].

In more severe cases, such as in cavitary or advanced/severe nodular bronchiectatic disease, instead of 3×/week, a daily regimen is recommended. For example, azithromycin (250 to 500 mg daily), rifampin (600 mg daily), and ethambutol (15 mg/kg daily) can be used along with parenteral amikacin or streptomycin in the initial treatment[64]. It is recommended that parenteral amikacin (10 to 15 mg/kg 3×/week) or streptomycin be used in the initial first 2–3 months of therapy for patients with cavitary, severe/advanced bronchiectatic MAC pulmonary disease, or macrolide-resistant MAC[63]. Patients with macrolide-susceptible MAC pulmonary disease should receive treatment for at least 12 months after culture conversion.

For patients with no positive outcome after 6 months with the guideline-based therapy, an amikacin liposome inhalation suspension (ALIS) treatment should be utilized in addition to their current treatment regimen. ALIS has demonstrated efficacy and safety when added to guideline-based therapy and is currently approved by the United States Food and Drug Administration (FDA) for treatment for refractory MAC pulmonary disease[65][66]. Surgery should also be considered from a risk/benefit perspective for certain patients who have not responded within 6 months[67]. The American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) recommend that surgical resection of MAC lung disease should be limited to patients with an adequate cardiopulmonary reserve to withstand partial or complete lung resection along with a multidrug treatment regimen. Additionally, surgery for MAC lung disease should b e performed in centers with

expertise on both the medical and surgical management of NTM diseases. Surgical resection of solitary pulmonary nodules from MAC is considered curative[62]. One study on treatment outcomes for 70 patients who had adjunctive pulmonary resections of NTM disease, had successful treatment outcomes for over 80% of its patients overall, although the post-operative outcomes and complications were relatively high. It is also important to note that the outcomes and the complications vary by the severity of the disease and type of surgery performed[67].

It is also important to note that the Clinical and Laboratory Standards Institute (CLSI) guidelines recommend antimycobacterial susceptibility testing (AST) for all slow-growing NTM infections, including MAC on all clinically significant isolates for proper antibiotic selection for a treatment regimen[68]. AST is performed for drugs which have a clear correlation between in vitro and in vivo outcomes. MAC has a clear correlation between the baseline macrolide susceptibility of the causative strain and the treatment outcomes with macrolide–ethambutol–rifampicin regimens[69][70]. The acquired macrolide resistance for MAC is from point mutations in the 23S rRNA(*rrl*) gene[71][72], while the acquired amikacin resistance is due to mutations in the 16S rRNA (*rrs*) gene[73]. The breakpoints for resistance are a MIC ≥ 64 µg/mL for parenteral amikacin and ≥128 µg/mL for amikacin liposome inhalation suspension (ALIS)[74]. Finding MICs above these breakpoints would require a cessation of intravenous or nebulized amikacin therapy[65]. The CLSI have also provided tentative breakpoints for linezolid and moxifloxacin, although in vitro–in vivo correlations have not been established[74].

5.2. Disseminated MAC Infection

Disseminated MAC infections spread to the rest of the body instead of staying localized to the pulmonary region. Through local multiplication and entry into the bloodstream, disseminated MAC infections may complicate the pulmonary disease by spreading to other organs and tissues. This disease is common in advanced HIV patients, patients with a history of immunosuppressive therapy, and other severely immunocompromised patients[19][75] (p. 13).

Treatment of MAC infection for patients with AIDS involves a combination of antimicrobial and antiretroviral therapy (ART) and may take more than 12 months. A dual therapy with a macrolide, azithromycin (500–600 mg daily), or clarithromycin (500 mg twice daily), combined with ethambutol (15 mg/kg daily) is initially used. In patients with failing ART, and when the mycobacterial counts in blood culture are high, a third agent (e.g., rifabutin) is added[76][77].

5.3. MAC Lymphadenitis

Nontuberculous mycobacterial (NTM) and MAC-specific lymphadenitis is transmitted through environmental sources and has a high risk of incidence among immunodeficient children[78][79]. The treatment of NTM or MAC specific lymphadenitis includes surgical excision and/or antimicrobial therapy depending on whether or not patients are good candidates for surgical excision. The increased risk of facial nerve damage can be a significant determining factor in whether to use antimicrobial therapy instead of surgical excision[80]. However, for patients with NTM lymphadenitis and no evidence of disseminated disease, surgical excision is the initial intervention and provides the optimal specimen for diagnostic testing. Antimicrobial therapy, with or without subsequent tissue excision, is the suggested method of therapy. An observational method of therapy is also sometimes utilized and involves monitoring patients for spontaneous disease resolution without the use of therapies. This prolonged course of observational treatment can be difficult to tolerate for some families[81]. The suggested regimen for antimicrobial therapy includes a macrolide in combination with ethambutol and/or rifampin. Azithromycin is the preferable macrolide for children due to it being more palatable and dosed once daily[62]. The duration for antimicrobial therapy of NTM or MAC specific lymphadenitis may take up to six months depending on the resolution of symptoms[82].

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