Advancing host-directed therapy for Mycobacterium avium infection: identification of drug candidates and potential host targets

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Chapter
1

General introduction

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Background & milestones NTM research

Tuberculosis (TB) is an ancient disease characterized by the presence of tubercles in tissues like the lungs and therefore historically described to be caused by ‘’tubercle bacilli’’. In 1882, Robert Koch isolated and identified the causative pathogen of TB and renamed it *Mycobacterium tuberculosis* (*Mtb*), which is the main cause of human infections due to *Mycobacterium* species (1). Following Koch’s discovery, other species of *Mycobacterium* were increasingly identified, which were referred to by several names, including ‘atypical mycobacteria’ and ‘nontuberculous mycobacteria’ (NTM). The earliest report of NTM was in the late 1880s when Alvarez and Tavil described the smegma bacillus (currently known as *Mycobacterium smegmatis*) found in human secretions (2). Nonetheless, it was already in 1868 in England, when Crisp observed seemingly TB in chicken (avian), later classified as *Mycobacterium tuberculosis avium* that mimicked the disease seen in humans, which was the first probable description of a bacterium now known as *Mycobacterium avium* (*Mav*) (**Figure 1**) (3). Koch initially stated that *Mav* was rather a variant of *Mtb* in animals, but more and more evidence became available to counteract his argument (3). According to Maffucci’s reports in 1890 and 1892, Rivolta suggested in 1883 and eventually also showed by experimental methods in 1889 that there was a difference between bovine TB and *Mav* found in chickens. In his reports, Maffucci described that *Mav* was definitely distinct from *Mtb* in the sense of cultural and pathogenic aspects, which was also confirmed by Cadiot, Gilbert, and Roger. However, since guinea pigs injected with *Mav* did not develop disease, *Mav* was believed not to cause disease in humans (3, 4). The development of improved culture techniques resulted in more accurate diagnoses of mycobacterial disease. In 1933, Branch reported the recognition of human-derived (pathogenic) *Mav* strains, and in 1943 Feldman et al. described a virulent *Mav* strain isolated from a patient with lung disease (5, 6). In 1949, a report by Cuttino and McCabe described a case of disseminated disease caused by a bacterial species, which was first named Nocardia intracellularis, later renamed to *Mycobacterium intracellulare* (*Min*) (7). Since *Mav* and *Min* are genetically very similar and not distinguishable by common laboratory examinations, they were together referred to as the *Mav* complex (MAC) (8). By 1953, more cases of MAC were described (9), and MAC was considered the most common cause of chronic lung infection due to NTM worldwide in the 1970s, which is still the case in many geographical regions. Interest in NTM increased in 1982, when disseminated infection, particularly caused by *Mav*, was dramatically more often observed in human immunodeficiency virus (HIV)-infected patients. While initially extremely rare, the recognition of *Mav* in patients with acquired immunodeficiency syndrome (AIDS) increased the number of disseminated cases strongly (10). Initially treated with solely *Mtb*-specific drugs, the implementation of clarithromycin in the 1990s marked a significant breakthrough in managing MAC disease. Meanwhile, the occurrence of MAC infections in AIDS patients was the first indication of the current knowledge that host immunity, specifically cell-mediated immunity, is critical for protection against MAC.

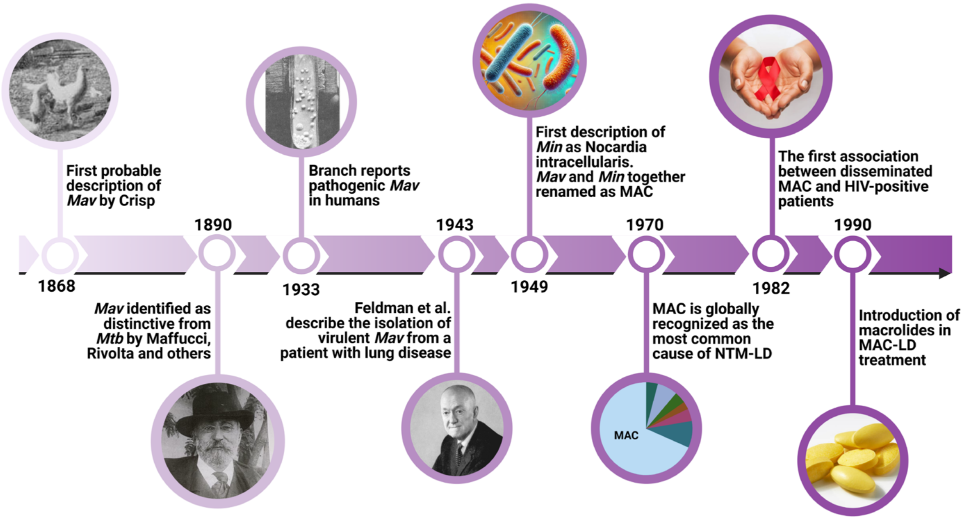

Figure 1. History of *Mav* (complex). *Mav*: *Mycobacterium avium*, *Mtb*: *Mycobacterium tuberculosis*, *Min*: *Mycobacterium intracellulare*, MAC: *Mycobacterium avium* complex, NTM: nontuberculous mycobacteria, LD: lung disease, HIV: human immunodeficiency virus. Created with BioRender.

MAC pathogenesis

Entry in, and recognition by host cells of the immune system

Given the airway-oriented nature of NTM infections, *Mav* may invade the mucosal barrier by interacting with bronchial epithelial cells to cause infection (11). Recognition and uptake of *Mav* by immune cells begins with the interaction of pattern recognition receptors (PRRs) on the cell surface that bind to pathogen-associated molecular patterns (PAMPs) to initiate a protective innate immune response against the mycobacteria. Characterization of the adhesions on *Mav* cell surface associated with the ability to interact with epithelial cells has identified the bacterial fibronectin attachment protein (FAP). FAP interacts with fibronectin to bind to integrin receptors on the surface of bronchial epithelial cells (12, 13). Once *Mav* reaches the alveolar space, it interacts with alveolar epithelial cells. Once recognized, *Mav* is taken up by epithelial cells requiring structural modifications of the cytoskeleton and proactive engagement of the cell (14). It is believed that *Mav*, by inducing biofilm formation and impairing the induction of an inflammatory response, may establish a chronic lung infection using the alveolar epithelial cells as a niche (15-17). While the mechanisms of escaping epithelial cells are unknown, it has been shown that *Mav* leaving epithelial mucosa has a different phenotype resulting in more efficient invasion of macrophages (18).

The mycobacteria may also directly, without interaction with epithelial cells, reach mononuclear phagocytes like monocytes and macrophages in the airways (19). There is a general consensus that macrophages represent the main reservoir of mycobacteria in the host (20, 21). Macrophages have a wide range of activation states with different functions, which can be broadly classified into two polar ends of the activation spectrum: pro-inflammatory macrophages (M1), involved in fighting infections, and anti-inflammatory macrophages (M2), which play a role in resolving inflammation and promoting tissue repair (22-24). In the healthy ‘’resting’’ state, human alveolar macrophages may possess an M1 or M2 phenotype (25, 26). During bacterial infections, however, host responses are skewing toward an M1 signature, which is associated with the control of acute infections. In contrast, the persistence of bacterial pathogens is linked to macrophage reprogramming to the M2 signature (27).

Macrophages express a wide variety of PRRs (28, 29), including Fc receptors, integrins, complement receptors (CR), C-type lectins, mannose and scavenger receptors. In addition to recognition, toll-like receptors (TLRs) are also involved in the induction of intracellular signaling cascades and pro-inflammatory responses. In particular TLR2, potentially by forming heterodimers with TLR1 and TLR6, plays a pivotal role in innate immune protection against *Mav* infection (30, 31). NTM, including *Mav*, express glycopeptidolipids (32), a major cell surface component that shields cell wall phosphatidyl-myo-inositol mannosides, thereby weakening recognition by TLR2 (33). Moreover, TLR6 and TLR9 are indispensable for managing *Mav* infection in mice (34, 35).

Host-pathogen interactions: macrophages vs. *Mav*

Once *Mav* is recognized, the macrophage membrane encapsulates and phagocytoses the mycobacteria, causing *Mav* to be targeted to cytoplasmic vacuoles called phagosomes. These phagosomes engage with the endosomal compartment to promote phagosome maturation (36). Phagosomes ultimately fuse with lysosomes that contain enzymes for bacterial killing (**Figure 2**). However, MAC can prevent its killing for example by impairing phagosome maturation by, using its secretory protein MAV_2941, interfering with vesicle trafficking and consequently fusion with lysosomes (37, 38). Moreover, mycobacterial membrane protein large 4 (MMPL4) participates in preventing phagosome maturation in *Mav*-infected cells by mechanisms not yet understood (39). Mycobacteria like *Mtb* and *Mycobacterium marinum* (*Mmar*) are known to be able to escape from the phagosome into the cytosol (40), where they can be targeted to autophagosomes to be degraded in a process called autophagy (or xenophagy) (41). In the same study, *Mav* remained phagosomal and showed no translocation to the cytosol, but the possibility of phagosomal escape has not been conclusively disproven.

In addition to direct recognition of *Mav*, macrophages can further be activated by IFN-γ released by CD4+ T helper 1 (Th1) cells induced by dendritic cells (DCs) via amongst others IL-12 (42). By presenting antigens and inducing T-cell responses, DCs link innate and adaptive immunity (43), in which the CD4+ T cell subset is essential for the host immunity against *Mav* (44, 45). Activation of the macrophage results in the TLR2-mediated production of pro-inflammatory cytokines, like IL-12, IL-23, and TNF (46, 47). IL-12 and IL-23 secreted by macrophages bind to their receptors on Th1 cells, promoting an increase in IFN-γ production. Furthermore, TNF induces apoptosis upon binding to its receptor TNFR1 (48). While most research indicates (TNF-mediated) host cell apoptosis as a host defense mechanism against mycobacterial, including *Mav*, infection (49-52), apoptosis can also be considered as a virulence mechanism of the bacteria as apoptotic macrophages have also been shown to result in the release and dissemination of *Mav* infection (53, 54). *Mav* expresses the MAV_2054 protein, which is known to induce macrophage apoptosis that can therefore be either host-protective or host-detrimental during *Mav* infection (55). Finally, macrophages generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) upon activation. While *Mav* tolerates RNS (56), ROS has been described to be involved in the killing of *Mav* by macrophages (57, 58). Taken together, while macrophages are the first-line defenders against *Mav* infection, bacteria can modulate host immune function to establish an intracellular replication niche that facilitates their replication and survival and evades immune detection.

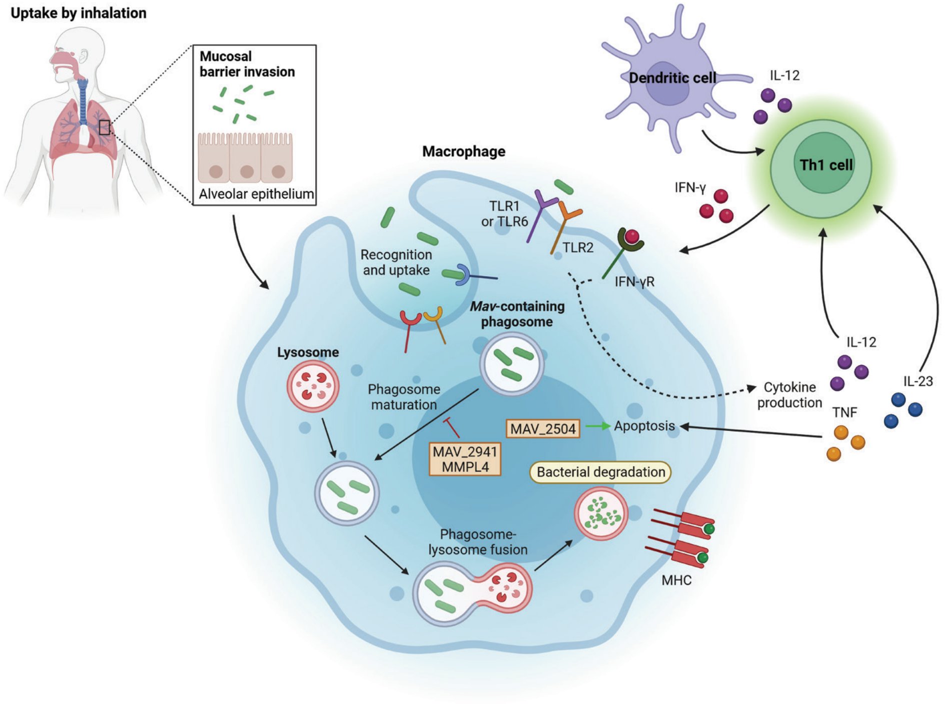

Figure 2. Phagocytosis and elimination of *Mav* by alveolar macrophages. Created with BioRender.

Mav exposure and risk factors

Environmental factors

While there is some evidence of human-to-human transmission (59), this type of transmission is extremely rare. Reasons may be the opportunistic nature of *Mav*, limiting infection in healthy individuals, and for example the lack of human-specific adaptations required for widespread transmission. Hence, it is believed that human disease due to *Mav* is acquired from environmental exposures. *Mav* and other NTM have been isolated from various environmental habitats, including both natural and treated water sources (e.g. drinking water distribution systems, hospitals, and household plumbing) (**Figure 3**), which are shared with humans and animals and have been associated with *Mav* disease (60-63). In addition to water, bacteria aerosolized as dust from potting soil has also been shown to be a risk factor for the development of disease due to *Mav* (64, 65). While the isolation of NTM from the environment is similar among different geographic areas (66, 67), higher risks for NTM infection and disease were identified in areas characterized by higher population densities and higher household education and income levels. These factors tend to cluster in more urbanized areas, which previously have been linked to NTM disease (68-70).

The major factor that permits the persistence of *Mav* and other NTM in environmental sources is their hydrophobic, lipid-rich outer membrane (71, 72). The hydrophobic characteristic of these bacteria enables their attachment to surfaces (73), which prevents bacteria from being washed out and allows them to form biofilms (74). Both the character of a thick cell wall and biofilm formation result in the increased tolerance of NTM to antibiotics and disinfectants.

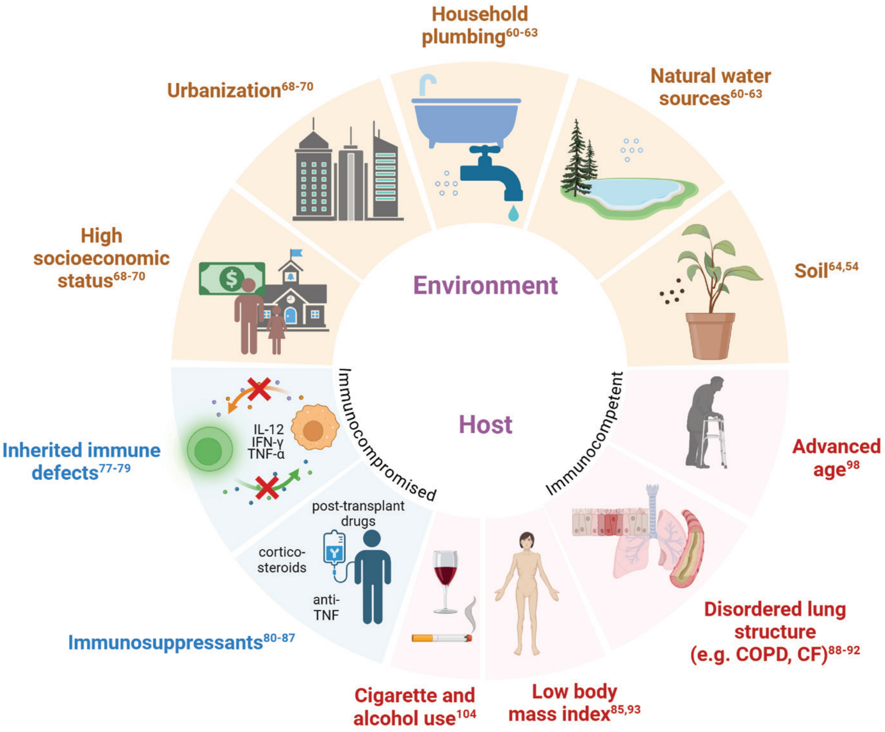

Figure 3. Environmental and host risk factors for MAC-LD. Created with BioRender.

Host factors

Due to their abundance, nearly everyone is presumed to be exposed to NTM, including *Mav*. Nevertheless, most people do not develop clinical signs or disease, indicating that host factors must also be involved in the outcome of exposure and infection. This was first reflected by the well-established association between disseminated NTM infections, particularly by *Mav*, in AIDS patients (75, 76), while the incidence of disseminated disease in this group was reduced by the administration of antiretroviral therapy. The key role of host immunity in the outcome of *Mav* infection is further supported by the development of lung disease (*Mav*-LD) in other immunocompromised phenotypes.

Inherited defects in the IFN-γ/IL-12 signaling pathways are known to be associated with increased susceptibility to mycobacterial infection and diseases, including *Mav* (77-79) (**Figure 3**), indicating that IFN-γ and IL-12 are both crucial elements in the host defense against NTM. Another pro-inflammatory cytokine induced upon *Mav* infection is TNF and its important role in controlling intracellular mycobacteria is shown by anti-TNF therapy; in several autoimmune diseases, targeting the TNF pathway with anti-TNF therapies, such as infliximab, adalimumab, and etanercept, increases the risk of the development of active TB (80), but also of *Mav* disease (81, 82). Similar to subjects receiving TNF blockers, patients receiving immunosuppressive drugs like corticosteroids (83-85), but also medication (e.g. tacrolimus) provided following organ transplantation (86), have higher rates of *Mav*-LD. Furthermore, individuals with solid tumors are at an elevated risk of developing lung disease caused by NTM, likely due to immune dysfunction associated with the disease or the immunosuppressive effects of chemotherapy (87).

However, *Mav* infections can also occur in hosts who are apparently healthy, without systemic immunosuppression, but often have (pre-existing) lung diseases or specific host characteristics (88). For example, cystic fibrosis (CF), an inherited disorder caused by mutations in the *CFTR* gene, leads to a reduced mucus layer and impaired mucociliary clearance, heightening the risk of the establishment of bacterial infection (89). Similarly, individuals with chronic obstructive pulmonary disease (COPD) or a history of pulmonary TB often have damaged lung structures, associated with a higher occurrence of *Mav*-LD (86, 90-92). Furthermore, lower body fat mass and BMI correlate with faster progression of *Mav*-LD (85, 93), which may be explained by the higher adiponectin and lower leptin levels expressed by fat cells, which have immunomodulatory effects (94-97). Furthermore, aging also increases susceptibility to *Mav* infection (98). This may be due to the simple fact that predisposing factors for *Mav* infection are more common with aging. However, independent of these underlying predisposing conditions, aging is also associated with immunosenescence that can affect key host defenses (99). With regards to gender, middle-aged (post-menopausal) females have a higher risk for *Mav*-LD (100-102), which may be related to the lower levels of estrogen as this has been shown to enhance the clearance of MAC in mice (103), although human data remain inconclusive (86, 87). In addition, middle-aged males with a history of smoking, alcohol use or aforementioned underlying lung diseases also have an increased risk for *Mav*-LD (104).

Although associations with some predisposing conditions are noticeably clear, predicting which individuals will develop *Mav* disease is not feasible. Nevertheless, factors that affect the host's susceptibility to MAC infection have enhanced our understanding of the pathogenesis of MAC, underscoring the significant role of the host's immune system in MAC infection.

Clinical presentations of *Mav* infection

Overall, *Mav* disease displays a range of clinical manifestations, from localized to systemic disease, largely influenced by the host's immune status and underlying risk factors. The most common site of *Mav* disease is the lung. *Mav*-LD can have two distinct forms (105, 106). Fibrocavitary lung disease, traditionally recognized as TB lung disease, is the severe form of *Mav*-LD and is characterized by areas of cavitation, pleural thickening, volume loss, and fibrosis, mostly in the upper lobes of the lung. This form is more commonly seen in middle-aged males. Without appropriate treatment, fibrocavitary disease progresses within a few years and can result in respiratory failure or destruction (107, 108). Alternatively, *Mav*-LD can present as nodular-bronchiectatic disease, which is more commonly observed in slender and middle-aged women, affecting mainly the middle lobe of the lung with small nodules and bronchiectasis (109). Although this form has a much slower progression rate, long-term follow-up is nevertheless warranted, as progression still may lead to death.

Another manifestation of *Mav* is disseminated disease (106), which develops upon infection via inhalation or ingestion (gastrointestinal route), and mainly occurs in severely immunocompromised (CD4+ T cells counts < 100/uL) AIDS patients (45). Presently, the occurrence of disseminated *Mav* disease in AIDS patients has become rare due to effective antiviral therapies (110), however, disseminated disease remains life-threatening if untreated (19). Treatment of *Mav* in these cases is often considered lifelong unless immune function is restored.

Furthermore, *Mav* infection in children frequently presents as lymphadenitis, most likely acquired via ingestion and which primarily affects the cervical lymph nodes. Since antibiotics are typically less effective, excision by surgery, with generally high success rates, is the treatment of choice (104, 106).

Finally, while mainly caused by rapidly growing NTM like *Mycobacterium fortuitum* and *Mycobacterium abscessus, Mav* can also cause localized infections involving the skin, soft tissues, or bones, often developed upon exposure to contaminated water, trauma, or surgical wounds (111). Diagnosis and treatment are often hindered due to the failure to recognize rare organisms as the cause of infection and the infrequent routine performance of mycobacterial cultures for surgical wound infections. Once diagnosed, patients frequently receive both drugs and undergo excisional surgery.

Challenges in the management of *Mav*

Diagnosis and epidemiology of *Mav*-LD

Based on the 2007 guidelines from the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA), the diagnosis of *Mav*-LD necessitates compatible clinical symptoms, compatible radiographic findings, and repeated microbiological detection of the species (104). The symptoms of *Mav*-LD, however, can be variable and non-specific such as chronic and recurring cough, and may also include weight loss, fever, chest pain, or fatigue. Since such symptoms usually overlap with underlying lung diseases mentioned above, it is often difficult to recognize them as symptoms of *Mav*-LD. Hence, it is essential to exclude other diseases such as TB for which IFN-γ release assay may assist (112). The radiographic features of *Mav*-LD are dependent on whether it is fibrocavitary or nodular-bronchiectatic. Radiographic features can be assessed with a chest X-ray or, if cavitation is not observed, a chest high-resolution computer tomography (HRCT) scan. Since these physical and radiographic features are not sufficient to distinguish *Mav*-LD from other lung disorders like TB, microbiological confirmation is the third criterion for accurate diagnosis and treatment decisions. Identification of the causative pathogen can be achieved by molecular assays like 16S rRNA sequencing using a minimum of three sputum specimens collected on separate days (113). In individuals who do not clearly meet the diagnostic criteria, a lung biopsy for diagnosing *Mav*-LD may be required (104). Diagnosing *Mav* infection requires the fulfillment of the equally important clinical, radiographic, and microbiologic criteria. Nevertheless, the diagnosis of *Mav* infection is often delayed due to non-specific symptoms, insufficient bacterial presence in sputum (114), resulting in late or incorrect treatment.

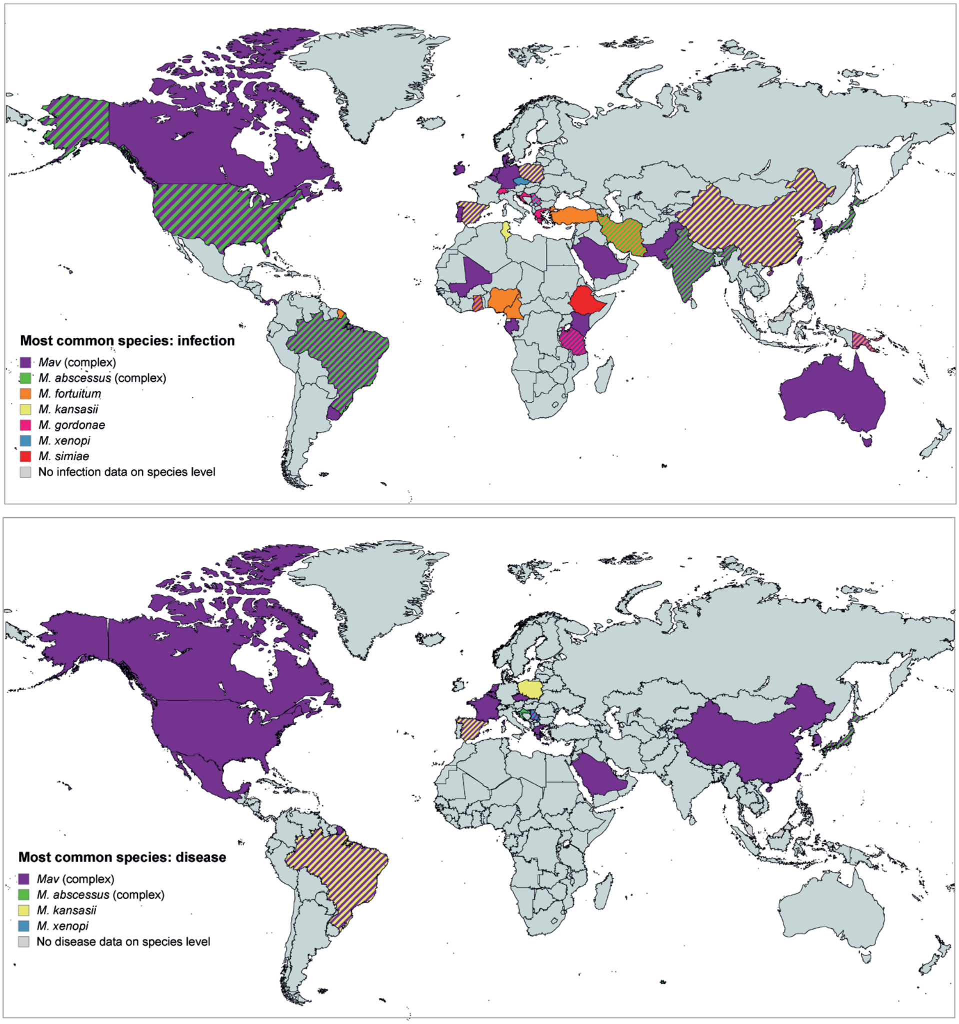

Figure 4. MAC is the most common species for NTM lung infection and disease worldwide. Created with MapChart.

Building on the challenges of diagnosing NTM, their reporting to public health authorities remains inconsistent. While NTM cases are seen in most industrialized countries, they are mandatorily reported in only a few states in the United States of America and Australia (115-117). The absence of a standardized global surveillance system limits the ability to accurately assess the burden of NTM to identify regional and national patterns that would allow insight into potential individual or environmental risk factors for *Mav* infection and concomitant disease. Nevertheless, by using microbiological data from centralized public health institutions and administrative claims, a comprehensive review by Dahl et al. revealed that across numerous studies from more than 18 countries, the majority reported an overall increase in NTM lung infection (82%) and in lung disease (66.7-78%, depending on the case definition criteria used) (118). The most frequently isolated NTM was MAC with increased trends of infection and disease in 78.9% and 83.9% of the studies, respectively. While other NTM species can be more frequent in certain countries, MAC predominates for both NTM lung infection and disease (**Figure 4**) in most geographical regions (45, 119).

The overall increase in prevalence of *Mav* has likely multifactorial causes. Obvious reasons may be increased awareness or improved microbiologic detection techniques (105). Moreover, the occurrence of *Mav* cases may also increase as the aging population, associated with specific risk factors for *Mav* disease, as described above, is growing in certain countries such as the Netherlands (69, 120, 121). Finally, it has been suggested that by inducing protective immunity, TB infection provides cross-protection to NTM and the increasing number of diseases caused by NTM may be due to a decrease in TB cases (122). However, regardless of the reasons for the increase in NTM, the increase in the number of *Mav* infections and disease highlights the importance of documentation of NTM cases in a standardized manner to monitor and better manage these complicated infections.

Unsatisfactory treatment outcomes for *Mav*-LD

The recommended treatment of *Mav*-LD involves a combination of antibiotics, including a macrolide like clarithromycin or azithromycin, along with companion anti-TB drugs like ethambutol and a rifamycin to prevent the emergence of macrolide resistance (104). The goal of treatment is clinical improvement within 3-6 months and negative sputum cultures for 12 months while on therapy (123). However, the extensive and intensive nature of these antibiotic regimens may also hamper treatment adherence and increase the risk of developing drug resistance, complicating effective disease management. Despite a consensus statement in 2018 regarding treatment outcome definitions (124), there is a lack of widely accepted definitions of treatment success. The lack of such definitions, combined with different disease severities as well as different drug regimens and dosages included in MAC-LD clinical trials has resulted in inconsistent treatment success rates. Based on various systematic reviews and meta-analyses, Kwon et al. reported relatively poor pooled treatment success rates of 32-65% for MAC-LD (125). Successful treatment of MAC infection with a macrolide-based therapy is associated with the development of macrolide-resistant MAC strains (6.6-20% of treated patients) (126), for which the treatment regimens are far less successful (sputum conversion rates of 15-36%) (125). Even after initial success, 50% of the treated patients had a relapse (refractory infection) resulting in a positive sputum culture while receiving the same treatment (127, 128). Hence, the overall treatment success rate of the combinatorial antibiotic regime for MAC-LD has been unsatisfactory.

Several factors can interfere with successful treatment, which includes the lack of adherence to guidelines-based therapy (129), lack of treatment compliance or tolerance (77), lack of response to the regimen, the emergence of macrolide resistance, and lack of effective treatment for macrolide-resistant disease (130-132). Moreover, MAC has also been associated with reinfection, which occurs in 25-48% of patients (133). Currently, only a small number of drugs, of which the majority have been repurposed, are evaluated in clinical trials for NTM-LD (134). This is likely a result of poor incentives, such as the lower profitability compared to communicable diseases like TB. Hence, new treatment strategies are urgently needed to potentiate, shorten and/or simplify current treatment strategies and improve treatment outcomes.

Antimicrobial susceptibility testing

For many years, antimicrobial susceptibility testing (AST) has long been conducted to predict the clinical effectiveness of antibiotics in treating NTM isolates. For MAC, the Clinical and Laboratory Standard Institute (CLSI) recommends using broth-based testing with both microdilution (multi-well plate) or macrodilution (radiometric BACTEC/Mycobacteria growth indicator tube (MGIT) system) (135, 136). However, unlike *Mtb* or rapidly growing mycobacteria (136, 137), it has long been known that for infections with slow growers like MAC, the correlation between *in vitro* susceptibility and good treatment outcomes for drugs is poor (138). Only *in vitro* susceptibility testing results for clarithromycin or clarithromycin-containing regimens correlated with *in vivo* efficacy (104), while the clinical response of MAC to ethambutol, rifampicin, and isoniazid using AST could not be predicted (139). Hence, the CLSI states that for MAC isolates only AST for clarithromycin is recommended.

The important discrepancies between AST results and the clinical response may stem from challenges in the laboratory process of AST, as well as the lack of standardized procedures and interpretation of the results. However, differential susceptibility *in vitro* versus *in vivo* may also be the result of specific bacterial behavior in a different setting. Suboptimal drug exposure and selection *in vivo* may differentially affect the interplay between tolerance and acquired resistance of bacteria interfering with susceptibility, which is not observed *in vitro* (140). Moreover, mycobacteria are known to be both intracellular and extracellular pathogens. Within cells, mycobacteria may adjust their metabolism or even become metabolically inactive (i.e. dormant) to prevent immune activation, possibly resulting in lower susceptibility to certain antibiotics that target active bacterial processes. Furthermore, *in vivo* bacteria might also reside in granulomas and biofilms which may affect their susceptibility to drugs. Hence, *in vitro* susceptibility testing of bacteria in conditions (more) resembling their physiological environment may improve the ability to translate the efficacy of drugs from *in vitro* to *in vivo*.

An alternative treatment strategy: boosting the host immune system

As reflected throughout this chapter, host immunity plays a crucial role in the outcome of *Mav* infection. Enhancing the host immune response to infection using host-directed therapy (HDT) may therefore be an alternative (adjunctive) treatment strategy to treat mycobacterial infections like *Mav*. HDT targets host processes to either reduce pathology caused by excessive inflammation or to enhance the host control of (intracellular) infection. The building knowledge on the host-pathogen interactions during *Mtb* infection has provided insights required for the development of HDT. By targeting host immunity rather than the pathogen, HDT has major advantages compared to conventional antibiotics including avoiding direct selective pressure on bacteria and thus reducing the risk of *de novo* development of drug resistance, but also the potential to shorten the duration or decreasing the dosage of current treatment regimens, which may reduce adverse drug effects. Although HDT offers the potential to treat infections, the development of HDT for *Mav* is yet limited. The potential of HDT to boost the macrophage's ability to fight MAC infection has been shown *in vitro* with for example cytokines like GM-CSF or IFN-γ (141). Acquiring a more thorough understanding of how the host and pathogen interact during MAC infection may allow the development of other, more potent, HDTs.

Outline of this thesis

Given the challenges of current antibiotic treatments for *Mav*, this thesis focuses on developing HDTs to combat *Mav* infections. To this end, human cell-based infection models were developed to identify HDT candidates that improved host control of intracellular *Mav* infection. Using these models, also the host response to *Mav* infection was studied, improving our fundamental understanding of *Mav* infection and further aiding the development of HDTs against *Mav*.

First, we provide a comprehensive literature overview of HDTs under investigation for mycobacteria in **chapter 2**. As the development of HDTs for *Mav* is limited, this review mainly reports HDTs that have shown efficacy in treating *Mtb* infections. Moreover, we also propose potential intracellular host factors that may be targeted by HDT to improve host infection control of mycobacteria. In **chapter 3**, we developed human cell-based infection models for *Mav*, using the phagocytic MelJuSo cell line and primary human macrophages, to enable the identification of potential HDT candidates that can improve the antimycobacterial activity of host cells against intracellular *Mav*. These models can also be used to study host-pathogen interactions during *Mav* infection. By using the primary human macrophage model in **chapter 4** and **chapter 5**, we identified amiodarone and phenothiazines as potential HDT candidates for *Mav* infection. We showed that amiodarone most likely acts by enhancing the host autophagy pathway to impair intracellular survival of *Mav,* while phenothiazines impair intracellular *Mav* survival by enhancing cellular ROS production and additional mechanisms that remained undiscovered. In **chapter 6**, we performed transcriptomic analysis of primary human macrophages infected with *Mav* alongside *Mtb* to compare the host response between *Mav* and *Mtb* and to facilitate the rapid extrapolation of relevant findings from *Mtb* to *Mav.* The results described in **chapter 6** not only enhance our understanding of the host transcriptomic response to both pathogens, but they also provide insights into host factors that may be exploited for the development of HDT for *Mav*. Finally, the findings of this thesis were summarized and discussed in **chapter 7.**

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