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Animal Models of Non-Tuberculous Mycobacterial Infections

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Abstract

The use of animal models has been essential in understanding the pathogenesis of and hosts immune response to tuberculosis, as well as testing potential antimicrobial compounds and vaccines. Experimental animals have also been used to study infections due to non-tuberculous mycobacteria (NTM). Because there are many different species of NTM capable of causing disease and they have varying degrees of virulence, developing animal models that are suitable for the diseases they cause isolated lung disease, skin and soft-tissue infections, and visceral extra pulmonary /disseminated disease is challenging. The goal of this review is to discuss the various animal models that have been used to study the pathogenesis of NTM infection as well as screening candidate antimicrobials, which are essential endeavors if better control of NTM infection is to be achieved.

Keywords Lung disease; Non-tuberculous; Mycobacterial infections

Introduction

The incidence and prevalence of non-tuberculous mycobacterial (NTM) lung disease are increasing in the United States and many parts of the world [1-8]. Among those >65 years old, the prevalence significantly increased from 20 cases per 105 in 1997 to 47 cases per 105 in 2007, a rate increase of 8.2% per year [1]. The precise reason(s) for the rising number of patients with NTM lung disease remains largely unknown. It has been speculated that the surge in numbers may be the result of several factors, including greater awareness and improved diagnosis, increased environmental exposure, iatrogenesis from use of inhaled medications, and person-to-person spread [9,10]. The purpose of this review is to discuss the various animal models that have been used to study the pathogenesis of NTM infection as well as screening candidate antimicrobials, which are essential endeavors if better control of NTM infection is to be achieved. We will neither discuss animal models for *Mycobacterium leprae* (nosologically not considered to be an NTM) nor *Mycobacterium avium* subspecies *paratuberculosis* (well known cause of disease in cattle but not man).

The most common NTM to cause lung disease belongs to the *Mycobacterium avium* complex (MAC) – historically comprised of *M. avium* and *M. intracellulare* – but with high-throughput gene sequencing, several more related species have been identified that are under the MAC umbrella, including *M. chimaera* [11-13]. In the United States, the next most common NTM to cause lung disease belongs to the *M. abscessus* complex – of which there are three known distinct species – *M. abscessus sensu stricto*, *M. massiliense*, and *M. boletti*. Depending on the region of the world, other NTM known to cause lung disease include *M. kansasii*, *M. xenopi*, *M. malmoense*, *M. szulgai*, *M. simiae*, *M. fortuitum*, and *M. chelonae* (list not exhaustive).

Animals Models have been Very Educational with Tuberculosis

Animal models – including mice, guinea pigs, rabbits, zebra fish, and non-human primates have been instrumental in understanding the pathogenesis and host immune response to tuberculosis (TB) [14,15]. Certain animal species have also been valuable in testing potential antimicrobial compounds and vaccines. Each model has its own advantages and disadvantages that include tractability, availability of reagents, cost, and ability to mimic various aspects of the human host immune response and pathology. The mouse model has been the most widely used due to the abundance of reagents and their relative low cost. The murine model has also been criticized due to differences in immune cellular responses and in granuloma architecture compared to human TB. Nevertheless, the mouse model has been instrumental in our early understanding of the host-protective immune response to TB. For example, knockout mouse studies showed many years ago that CD4+ cells, tumor necrosis factor-alpha (TNFα), interferon-gamma (IFNγ), and interleukin-12 (IL-12) were essential components of anti-TB immunity, which have been subsequently shown to be true in individuals who are infected with HIV, prescribed anti-TNFα antagonists, and possess genetic defects in the IFNγ-IL-12 axes, respectively. The worldwide increase in NTM disease has forced the need to better understand its pathogenesis – which remains inadequately characterized. The unmet medical needs of NTM patients are urgent and require a greater understanding of the cellular phenotypes which control infection and disease in order to develop new or repurposed active compounds, and anti-infective and prophylactic vaccines, with the ultimate goal of curing or at least mitigating this epidemic. Given the usefulness of animal models in understanding the pathogenesis and host-immune response to TB, it is likely that animal models will also prove to enhance our understanding of NTM infections. The goal of the paper is to review the different animal models that have been investigated with NTM infections.

Routes of Infection in Animal Models

Various routes of infection have been described in infecting experimental animals with mycobacteria including intravenous, intraperitoneal, intratracheal, intranasal, intragastric, and aerosolization [14-17]. However, for human NTM lung disease, the two most likely routes of natural infections are inhalation of NTM-infected aerosol and aspiration of NTM that have colonized the upper aerodigestive tract. Two lines of experimental evidences support inhalation of droplet nuclei as a mechanism for infection into the lungs. First, it has been shown that natural bioaerosols, e.g., rivers and streams, can generate droplet sizes on the order of 10–150 μm in diameter. Upon drying, droplets that are 10–50 μm will shrink to sizes that are <5 μm, small enough to be inhaled directly into the alveoli. Man-made aerosols such as that from hot tubs, spas, and showers have very active bubble generation and thus, are likely to produce droplets of similar and even smaller sizes. Second, Falkinham et al. [8] showed that because NTM possess hydrophobic properties, they are highly concentrated on droplet surfaces, up to an order of ~5000-fold greater in concentration than the water source from which the aerosols were generated.

Attempts to develop a pulmonary model of *M. abscessus* by exposing mice to $1.0x10^{11}$ *M. abscessus* by aerosolization resulted in a low level of infection in severe combined immunodeficiency (SCID) mice and granulocyte monocyte-colony stimulating factor knockout (GM-CSF-/-) mice (unpublished data). Attempts to deliver $1.0x10^{12}$ *M. abscessus* were not technically feasible due to the inoculum becoming a pasty material that could not be aerosolized [18,19]. An intratracheal infection in GM-CSF knockout [20] meets the requirements for a preclinical antimycobacterial *M. abscessus* model although it requires sedation of the animals during the infection procedure. In addition, the process of intratracheal infection may cause perforation of the trachea and result in seeding of the bacteria into the vessels surrounding the trachea, resulting in an intravenous infection.

The stomach was traditionally thought to be a barrier to mycobacteria, but it was shown that virulent *M. avium* strains could infect mice orally and be found in gut lymphoid tissues [21,22]. If the beige mutant in the C57BL/6 mouse was used, this was amplified [21,22]. Our laboratory has also found that SCID mice infected orally with *M. abscessus* resulted in a progressive infection (Ordway D et al., [18] unpublished observations). Thus, it is plausible that NTM can survive in the stomach following ingestion – particularly if they are being treated with acid suppressive medications – and in those with gastroesophageal reflux, aspirate viable NTM into the lungs.

Animal Models of NTM Infection

Mouse models

In humans, the two major categories of NTM diseases are isolated lung disease and extrapulmonary-disseminated disease. Individuals in the latter group are typically more immunocompromised. Thus, the mouse strain that is chosen should depend on the disease of interest although it is difficult to mimic chronic NTM disease that is exclusively isolated to the lungs. Since NTM are generally less virulent than *Mycobacterium tuberculosis* (MTB), the ability to induce a productive and sustained infection in a mouse strain becomes a factor as well (Table 1). Prior studies have revealed that most immunocompetent mouse strains (e.g., C57BL/6) serve as excellent models for the more virulent MAC species but are rapidly cleared when infected with *M. abscessus* [19].

Mouse strain	NTM used Route of infection	Productive infection (organ)	Progressive or persistent infection/disease	Reference
C57BL/6	*M. abscessus* Aerosolization (HDA and LDA)	Yes, HDA (lungs & spleens) No, LDA	No	39
C57BL/6	*M. avium* Aerosolization (~10^5/mouse)	Yes (lungs & spleens)	Yes, followed up to 12 weeks	38
129Sv	*M. avium* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes	30
BALB/c	*M. abscessus*-R IT (10^4/mouse)	Yes, IT, (lungs & spleen)	No	42
BALB/c	*M. avium* Aerosolization (~10^5/mouse)	Yes (lungs & spleens)	Yes, followed up to 12 weeks	38
Ob/Ob	*M. abscessus* Aerosolization (HDA and LDA)	Yes, HDA (lungs & spleens) No, LDA	No	39
Cystic fibrosis mouse	*M. abscessus* R & S morphotype IT ($1.6x10^6$/mouse)	Yes	Yes, but followed for up to only 14 days	41
iNOS-/-	*M. avium* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes	30
iNOS-/-	*M. avium* IV (10^6/mouse)	Yes (liver)	Yes, followed for up to 120 days	34
iNOS-/-	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, & liver)	No, resolution of infection by 20-40 days	19
Cybb-/-	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	No, resolution of infection by 40 days	19
TNFα receptor-/-	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	No, resolution of infection by 40 days	19
TNFα p55 receptor-/-	*M. avium* IV (10^6/mouse)	Yes (lungs & spleens)	Yes, followed for up to 5 weeks	56
MyD88-/-	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes, but decreasing with time	19
C3HeB/FeJ	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes, but decreasing with time in the lung	19

Beige	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	No, resolution of infection by 20-40 days	19
Beige	*M. avium* Aerosolization (~10^5/mouse)	Yes (lungs & spleens)	Yes, followed up to 12 weeks	38
Beige	*M. avium* IV (10^6/mouse)	Yes (lungs, spleen, liver, gut)	Yes, followed up to 60 days	36
GKO	*M. abscessus* Aerosolization	Yes, LDA or HAD (lungs & spleen)	Yes	39
GKO	*M. abscessus* IV (10^6/mouse)	Yes (lungs, spleen, liver)	Yes, but decreasing with time	19
GKO	*M. massiliense*	Yes (lungs, spleen)	Yes, with epidemic strain of M. massiliense	40
GM-CSF-/-	*M. abscessus* Aerosolization (10^6/mouse)	Yes (lungs, spleen)	Yes in lungs Resolution in spleen by 4 months	20
GM-CSF-/-	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes, followed for up to 40 days	19
Nude	*M. abscessus* IV (10^6-10^8/ mouse)	Yes (lungs, spleen, liver, kidneys)	Yes, followed up to 60 days	43
Nude	*M. abscessus* IV (10^6/mouse)	Yes, (lungs, spleen, liver)	Yes	19
Nude	*M. avium* Aerosolization (~10^5/mouse)	Yes (lungs & spleens)	Yes, followed up to 12 weeks	38
SCID	*M. abscessus*-R IT (10^4/mouse)	Yes (lungs, spleen)	Yes with the R strain but not the S strain	42
SCID	*M. abscessus* IV (10^6/mouse)	Yes (lungs, spleen, liver)	Yes, followed for up to 40 days	19
HDA=~1000 bacilli/mouse; LDA=~100 bacilli/mouse; R=rough morphotype of *M. abscessus*; S=smooth morphotype of *M. abscessus*				

Table 1: Mouse strains used for NTM infections.

MAC species

The role nitric oxide (NO) plays as a host-defense molecule in human TB is controversial [23,24]. In contrast, it is well established that NO plays a host protective role against MTB in murine macrophages and in mice *in vivo* [25-30]. While it would seem that NO would also have anti-NTM properties in mice, it was found that the inducible nitric oxide synthase knockout (iNOS-/-) mice were better able to control an intravenous infection with *M. avium*; i.e., with a less virulent strain of *M. avium*, there was lower mycobacterial burden in the liver, spleen, and lungs of the iNOS-/- mice compared to wildtype 129Sv mice 30. With a more virulent *M. avium* strain, the iNOS-/- mice also had lower burden of *M. avium* in the spleens and lungs but for unclear reason had modest increase in *M. avium* numbers in the liver compared to wildtype mice. Furthermore, IFNγ + TNFα treatment of bone marrow-derived macrophages from both wildtype and iNOS-/- mice decreased intracellular *M. avium* burden, indicating that the anti-NTM effect of the two cytokines is not mediated by production of NO. iNOS-/- mice also had greater IFNγ production, greater granuloma formation, and increased CD4+ T cell number. The implication is that NO can inhibit IFNγ production; consistent with prior work showing that NO has inhibitory effect on IFNγ production by TH1 cells [31]. The authors also speculated that since NO appear to inhibit granuloma formation and mouse cells make a lot more NO than human cells – perhaps the reason mouse granulomas are less organized is because mice produce greater amounts of NO [30]. Another speculative mechanism by which NO may promote NTM growth is that since reactive oxygen species have been shown to be effective in inhibiting growth of *M. avium* [32], NO combining with superoxide to form peroxynitrite (ONOO-) reduces the availability of superoxide. But the fundamental reason why NO is effective against MTB and not against NTM remains a mystery. While the aforementioned study implicates IFNγ as a host-protective cytokine, it has also been shown that virulent *M. avium* are able to grow in mice in spite of a dominant TH1-IFNγ responses [33]. Subsequent studies also established a superior fibrotic response in the lungs of *M. avium*-infected iNOS-/- mice [34].

Gonzalez-Perez et al. [35] infected BALB/c mice with a high dose of *M. avium* subspecies avium or *M. colombiense* intratracheally and found high expression of TNFα and iNOS as well as granuloma formation with rapid clearance of the NTM; during the later stages of the infection, expression of anti-inflammatory cytokines resulted in resolution of the lung consolidation [35]. While one may infer from this study that iNOS and NO are host-protective against *M. avium* – a notion that is opposite to the aforementioned study – it is important to note that the mere presence of iNOS and resolution of infection does not necessarily indicate a cause-and-effect; furthermore, the subspecies of *M. avium* differ between the two relevant studies [30,35].

The beige mutation in the C57BL/6 mice has been used to study *M. avium* pathogenesis and compound screening [17]. Dissemination of *M. avium* from the gut was faster in the beige mice than the wild type C57BL/6 mice [36]. Production of IFNγ was similar between the two mouse strains infected with *M. avium*. However, the beige mice had a defect in the influx of neutrophils to the site of infection as transfusing neutrophils mitigated their susceptibility, and neutrophil depletion studies with wildtype C57BL/6 mice demonstrated increased susceptibility [36]. Subsequent studies but not with the beige mice revealed that defect in CXCR2 chemokine signaling impaired the early and rapid recruitment of neutrophils with *M. avium* infection [37].

Andrejak et al. [38] compared BALB/c, C57BL/6, nude, and beige mice in a *M. avium* strain Chester aerosol infection and compared *in vivo* susceptibility to antimicrobials. Nude mice were the most sensitive to *M. avium* but the efficacy of treatment was most noticeable in *M. avium*-infected BALB/c mice. They found that clarithromycin-rifampin-ethambutol combination was superior to the moxifloxacin-rifampin-ethambutol regimen.

Rapidly-growing mycobacterial species

The rapidly growing mycobacteria (RGM) – historically defined as visible detection of NTM colonies on solid medium <7 days after inoculation – have also been used to infect mice. The most clinically relevant RGM to cause human lung disease belongs to the *M. abscessus* complex, comprised of *M. abscessus* sensu stricto, *M. massiliense*, and *M. boletti*. Because *M. abscessus* sensu stricto has a functional erm [39] gene, inducible resistance to macrolides may be seen. In contrast, *M. massiliense* does not have a functional erm [39] gene and thus its sputum conversion rate to negative is significantly better than response seen with *M. abscessus* sensu stricto (85% vs 15%, respectively). Our laboratory characterized the lung immune responses in mice infected with *M. abscessus* [18]. C57BL/6 and leptin-deficient (Ob/Ob) mice challenged with a low-dose aerosol (LDA, ~100 bacilli per mouse) of *M. abscessus* did not develop an infection. However, when challenged with a high-dose aerosol (HDA, ~1,000 bacilli per mouse), C57BL/6 and Ob/Ob mice developed an established infection and a pulmonary immune response consisting of an early influx of IFNγ+CD4+ T cells; this immune response preceded the successful clearance of *M. abscessus* in both strains of mice, although mycobacterial elimination was delayed in the Ob/Ob mice. In contrast to the C57BL/6 and Ob/Ob mice, IFNγ knockout (GKO) mice challenged with a LDA or HDA of *M. abscessus* showed a progressive lung infection despite a robust influx of T cells, macrophages, and dendritic cells, culminating in extensive lung consolidation. Furthermore, with HDA challenge of the GKO mice, emergence of IL-4- and IL-10-producing CD4+ and CD8+ T cells were seen in the lungs. Thus, IFNγ is critically important in the host defense against *M. abscessus*.

Shang et al. infected GKO mice with a glutaraldehyde (GTA)-sensitive strain of *M. massiliense* with one that was resistant to the disinfectant and responsible for a nosocomial outbreak [40]. Compared to the GTA-sensitive strain, the GTA-resistant strain of *M. massiliense* replicated more efficiently in mice; the greater burden of the GTA-resistant *M. massiliense* was associated with delayed influx of TNFα+ CD4+ and CD8+ T cells, increased number of T regulatory cells, progressive infection, and extensive lung consolidation.

Caverly et al. [39] intratracheally infected wildtype and cystic fibrosis mice with *M. abscessus* suspended in fibrin plugs and found greater burden of mycobacteria in the lungs than systemically three and 14 days after infection. Interestingly, infection with the rough morphotype of *M. abscessus* resulted in greater number of neutrophils in the bronchoalveolar lavage in both mouse strains. Spontaneous *in vivo* conversion from the smooth to the rough morphotype occurred in ~20% of the *M. abscessus*.

DeGroote et al. [20] infected GM-CSF-/- mice with aerosolized *M. abscessus* delivered into the laryngeal vestibule using a microsprayer device. Upon initial infection with $5x10^5$ *M. abscessus* organisms per mouse, there was an initial rise in the number in the lungs and then a downward trend over the next several weeks, followed by a steady rise over the next two to four months such that the number of CFU recovered at four months was close to the number recovered soon after the initial infection. In contrast, *M. abscessus* was cleared from the spleens by four months. Lung pathology revealed peribronchial and perivascular lymphocytic inflammation with areas of bronchiectasis.

Recently, our laboratory found that even mice with specific defects in innate or acquired immunity infected with $1x10^6$ *M. abscessus* intravenously were able to control the infection; these mouse strains included the beige mice (dominant TH2 immunity), iNOS-/- mice, Cybb-/- mice (devoid super-oxide generating enzyme), TNFα receptor-/- mice, C3HeB/FeJ mice (notable for displaying necrotic granulomas with MTB infection), GKO mice, and the MyD88-/- mice [19]. After 40 days of infection there were still viable *M. abscessus* – albeit at reduced levels from the initial inoculum – in the lungs of the C3HeB/FeJ, GKO, and MyD88-/- mice, whereas viable mycobacteria were undetectable in the other mouse strains. Furthermore, the GKO-/- and MyD88-/- mice also still had viable but reduced levels of *M. abscessus* in the spleen and liver after 40 days of infection [19]. These findings in the GKO mice infected with *M. abscessus* intravenously are in contra-distinction to our previous finding that GKO mice infected with *M. abscessus* by aerosolization resulted in a progressive infection followed for up to 60 days [18], suggesting that the route of infection is critically important in the host-immune response.

In contrast, the SCID mice, nude mice, and GM-CSF-/- mice infected intravenously with *M. abscessus* had sustained or progressive bacterial burden 19. The ability of SCID, nude, and GM-CSF-/- mice to support sustained NTM growth points to an important role of T cells and GM-CSF dependent cell phenotypes for protective immunity against NTMs. Byrd and Lyons [41] found that SCID mice had sustained infection (up to 28 days of followup) following intratracheal infection with the rough morphotype of *M. abscessus* but there was relatively rapid clearance when the mice were infected with the smooth morphotype. Since these three immunodeficient mouse strains as well as the GKO and MyD88-/- mice had a significant number of viable *M. abscessus* at Day 40 after infection, the antimycobacterial activity of clarithromycin, clofazimine, bedaquiline, clofazimine-bedaquiline, ciprofloxacin, and amikacin were tested in *M. abscessus*-infected GKO and SCID mice [19]. The most effective drugs in ascending order of efficacy were clarithromycin (less effective), clofazamine, amikacin, bedaquiline, and clofazamine-bedaquiline (more effective); ciprofloxacin was not effective. Interestingly, clarithromycin was effective after five days of treatment but not effective with longer treatment times, suggesting the possibility of inducible resistance to clarithromycin by an active erm41 gene product. Nevertheless, the advantage of infecting severely immunodeficient mice with *M. abscessus* is that the higher bacterial burden seen allows the potential to detect significant reduction in *M. abscessus* with drug treatment [19,42].

Intravenous infection of nude mice with 10^6-10^8 *M. abscessus* per mouse resulted in a static high level of bacterial burden [42]. The *M. abscessus*-infected nude mice were treated for two months with bedaquiline but – in contrast to the previous study – this did not significantly reduce the bacterial burden in the lungs and spleens [42]. The reason for this difference is most likely due to differences in *M. abscessus* strains used but may also be due to differences in mouse strains employed (GKO and SCID vs. nude mice). Additionally, the longer times of bedaquiline monotherapy in the latter study could have selected out naturally resistant *M. abscessus* strains or perhaps induced resistance to bedaquiline.

Another advantage seen with *M. abscessus* infection of the severely immunocompromised mice (SCID, nude, and GM-CSF-/-) was the presence of foamy cells to the lungs after forty days of infection, a cellular phenotype commonly seen in the histopathologic specimens of human NTM lung disease [19]. Another aspect of pulmonary NTM infection in humans is the development of non-necrotic and necrotizing granulomas, both of which could only be reproduced in

the SCID mice. It is very likely though, that changing parameters such as the virulence of the *M. abscessus* strain, inoculating dose, and/or duration of infection, the GM-CSF-/- and nude models could potentially produce necrotizing granulomas as well.

Guinea pigs

Buruli ulcer is a tropical, chronic, necrotizing disease of the skin, soft tissues, and occasionally bone caused by Mycobacterium ulcerans. Cutaneous infection of guinea pigs with *M. ulcerans* resulted in ulcers that on microscopy showed necrosis, acute inflammation, and high bacterial load [43]. Importantly, there was over time resolution of the ulcers, which mimics the spontaneous healing of Buruli ulcers.

Our laboratory also infected guinea pigs with *M. abscessus* by aerosolization and quantified the bacterial burden [18]. Infected guinea pigs showed peak bacterial load in the lungs, spleen, and regional mediastinal lymph nodes at Day 30 but were able to clear both HDA and LDA infections by Day 60 although the histopathology was more severe with the HDA infection.

Rabbits

Literature from over 50 years ago cites the use of rabbits with joint infections with atypical mycobacteria [44,45]. Thirty-five years ago, Meissner reported infecting rabbits as well as guinea pigs and hens with MAC organisms and noted large inoculum were required to create pathologic lesions in the animals [46]. More recently, eye infections to various NTM have been performed on rabbits [47,48]. Immune response of rabbits have also been examined to NTM antigens [49]. Otherwise, we are not aware of lung or systemic infections of rabbits with NTM.

Zebra fish

Zebrafish are increasingly utilized as a tractable infection model to study immunopathogenesis and host-immunity of vertebrates. Zebrafish embryos infected with various inoculation size of *M. marinum* – which shares virulence factors with *M. tuberculosis* – can result in acute infection, chronic infection with caseating granulomas, and one that closely resembles human latent infection [50]. Zebrafish embryos injected with either the rough or smooth strain of *M. abscessus* into their caudal vein resulted in chronic or acute infection, respectively [51]. Using a zebra fish model to study the pathogenesis of *M. marinum*, Huang and co-workers showed that compared to wildtype *M. marinum*, mutants deficient in the cell wall lipids phthiocerol dimycocerosates (PDIMs) and phenolic glycolipids (PGLs) induced more apoptosis and had a delay in the recruitment of eosinophils [52]. They hypothesized that PDIMs/PGLs are virulence factors through their capacity to inhibit host-protective apoptosis and recruitment of eosinophils promoted *M. marinum* growth. Oksanen et al. showed that intraperitoneal BCG vaccination of adult zebrafish afforded greater protection from subsequent high-dose *M. marinum* infection [53]. Additional models such as the embryonic zebrafish test system have been developed to assay *M. abscessus* for rapid compound screening [54].

Conclusion

NTM infections are becoming an emerging problem worldwide. To confront the morbidity and mortality associated with these difficult-to-treat pathogens, multiple laboratories are focused on developing pre-clinical models to understand the pathogenesis as well as screen new or repurposed compounds to combat these pathogens. One challenge with investigational studies of NTM is that there are multiple pathogenic species that cause human disease with likely differences in virulence not only between species but perhaps also between different strains within a species. A large number of different mouse strains have been studied with NTM infection but much fewer other animal models employed. While MAC organisms are more capable of establishing a productive infection in immunocompetent mice, *M. abscessus* requires more immunocompromised mice to result in such infection. It is clear that while the more severely immunodeficient mice can result in a sustained or progressive infection, the route and likely the morphotype of the NTM impact whether such sustained infection is achieved. If the goal is to study the pathogenesis and host-immune response of NTM lung disease, it would seem logical to infect the mice via aerosolization or intratracheal instillation. However, if the goal is to screen the efficacy of potential antimicrobial compounds, then a productive NTM infection is more paramount and perhaps which mouse strain or which route of infection is less of a factor. For studying extra-pulmonary and disseminated NTM disease, using a mouse strain the best mimics the underlying human immunodeficiency of interest seems most appropriate.

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