Advancing Translational Science for Pulmonary Nontuberculous Mycobacterial Infections

A Road Map for Research

Shelby Daniel-Wayman[1], Getahun Abate[2], Daniel L. Barber[3], Luiz E. Bermudez[4,5], Rhea N. Coler[6,7], Michael H. Cynamon[8,9], Charles L. Daley[10], Rebecca M. Davidson[11], Thomas Dick[12,13], R. Andres Floto[14], Emily Henkle[15], Steven M. Holland[16], Mary Jackson[17], Richard E. Lee[18], Eric L. Nuermberger[19,20], Kenneth N. Olivier[21], Diane J. Ordway[17], D. Rebecca Prevots[1], James C. Sacchettini[22], Max Salfinger[23,24], Christopher M. Sassetti[25], Christine F. Sizemore[26], Kevin L. Winthrop[15,27], and Adrian M. Zelazny[28]

[1]Epidemiology Unit, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, [3]T Lymphocyte Biology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, [16]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, [21]Laboratory of Chronic Airway Infection, Pulmonary Branch, Division of Intramural Research, National Heart, Lung, and Blood Institute, and [28]Microbiology Service, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland; [2]Division of Infectious Diseases, Allergy, and Immunology, Department of Internal Medicine, Saint Louis University, Saint Louis, Missouri; [4]Department of Biomedical Sciences and [5]Department of Microbiology, Oregon State University, Corvallis, Oregon; [6]Infectious Disease Research Institute, Seattle, Washington; [7]Department of Global Health, University of Washington, Seattle, Washington; [8]Veterans Administration Medical Center, Syracuse, New York; [9]State University of New York Upstate Medical Center, Syracuse, New York; [10]Division of Mycobacterial and Respiratory Infections, Department of Medicine, [11]Center for Genes, Environment, and Health, and [23]Mycobacteriology and Pharmacokinetics Laboratories, National Jewish Health, Denver, Colorado; [12]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [13]Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, New Jersey; [14]Department of Medicine, University of Cambridge, Cambridge, United Kingdom; [15]OHSU-PSU School of Public Health and [27]Division of Infectious Disease, Department of Medicine, Oregon Health & Science University, Portland, Oregon; [17]Mycobacteria Research Laboratories, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado; [18]Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, Tennessee; [19]Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; [20]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; [22]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas; [24]College of Public Health, University of South Florida, Tampa, Florida; [25]Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, Massachusetts; and [26]Tuberculosis, Leprosy, and other Mycobacterial Diseases Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland

Nontuberculous mycobacterial pulmonary disease (NTM PD) may be chronic, requiring lengthy and complex regimens over many months to years. After treatment, patients are often reinfected, with disease recurrence. NTM PD prevalence was estimated at 86,000 cases in the United States in 2010, surpassing pulmonary tuberculosis (TB), which was 9,272 in 2016 and declining; multiple studies in different U.S. and Canadian populations have indicated increasing prevalence since the mid-1990s (1). Increasing prevalence of NTM PD has also been documented in Australia, Asia, and Europe (2). Clinical care standards (3) would benefit from controlled clinical trials. To identify critical gaps in diagnosis, treatment, and prevention of NTM PD, the National Institute of Allergy and Infectious Diseases convened a workshop on September 26, 2017, bringing together diverse experts in mycobacterial disease. This report summarizes the key questions in host–pathogen interactions, molecular epidemiology and diagnostics, and vaccine/therapeutics. These key focus areas may help guide biomedical research efforts into this growing concern.

Development of Preventive and Treatment Strategies Will Require a More In-Depth Understanding of Host–Pathogen Interactions

Host and pathogen factors are known to contribute to NTM PD. Certain body morphotypes and sex (4), structural lung abnormalities, genetic disorders affecting mucociliary clearance, and the use of immunosuppressive drugs, such as steroids and tumor necrosis factor (TNF)-α blockers, are associated with a higher risk of NTM PD (5). Impaired ciliary function predisposes to NTM disease (6). How airway clearance affects mycobacterial attachment and invasion must be characterized. *Mycobacterium avium* subsp. *avium* and *Mycobacterium abscessus* subsp. *abscessus* infection of human bronchial epithelial cells cultured at the air–liquid interface lead to down-regulation of cilia genes and up-regulation of cholesterol biosynthesis and proinflammatory markers, including IL-32 (7), suggesting significant

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Correspondence and requests for reprints should be addressed to D. Rebecca Prevots, Ph.D., M.P.H., National Institute of Allergy and Infectious Diseases, NIH, 8 West Drive, MSC 2665, Bethesda, MD 20892. E-mail: rprevots@niaid.nih.gov.

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immune signaling from respiratory epithelium *per se*. Further investigation into how contact between NTM and lung epithelia triggers immune responses, through the addition of immune cells to the air–liquid interface model, may identify key mechanisms that can be modulated. Distinct immune pathways may be relevant to NTM infection in cystic fibrosis (CF); the high prevalence of NTM PD among persons with CF (8) may facilitate studies of host factors that vary between those who do and do not have NTM PD. Interestingly, patients receiving TNF-α inhibitors (5) for inflammatory conditions have an elevated risk of NTM infection. Further research in all of these areas will help identify host biomarkers to identify disease activity.

Vaccines that are able to limit infection might have an ameliorative effect on NTM PD in susceptible hosts. Early studies in mice identified candidate antigens and approaches (9, 10). It is unclear whether shared antigens can elicit protection against the diverse NTM that cause disease (11). Reports of increased incidence of extrapulmonary NTM in Sweden and Finland after the end of bacillus Calmette-Guérin (BCG) vaccination to protect against TB suggest that BCG may provide collateral protection against extrapulmonary NTM (12, 13). However, no evidence is available regarding its effect on pulmonary NTM. Considering the different factors implicated in pulmonary versus disseminated NTM, a pulmonary vaccine, presumably inhaled, might be of interest to explore in NTM PD. Mycobacterial biofilms or microaggregates have been demonstrated in the lungs of patients with *M. abscessus* infections (14). *M. avium* subsp. *hominissuis* (15) biofilm appears to be important for invasion and infection (15–18). Therefore, we need a better understanding of biofilms, including their structure, physiology, and how concomitant pathogens interact with each other. For example, some evidence exists that *M. abscessus* subsp. *abscessus* is able to degrade quinolone signals from *Pseudomonas aeruginosa* (19).

Biofilms may affect susceptibility to antibiotics, persistence, and antigen exposure, which in turn may affect immune responses and pathology. We need animal models that reflect critical characteristics of the pathogens in these unique niches to explore and vet promising drug candidates.

Pathogen Diversity Impacts Disease Dynamics, Diagnosis, Drug Resistance, and Treatment Outcomes

Significant genotypic and phenotypic diversity exists across and within NTM species. *M. avium* strains responsible for pulmonary infections may differ from those that lead to disseminated disease (20). The relative prevalence of NTM species varies with the underlying lung disease, such as CF or chronic obstructive pulmonary disease (COPD): for example, in Scotland, 68% of patients with CF had *M. abscessus* compared with 5% of patients without CF; in Japan, 24% of patients with *M. abscessus* had COPD versus 5% of patient with *M. avium* complex (MAC) (21, 22). A publicly accessible, well-characterized reference panel of NTM species is a critical first step in the systematic study of these organisms in a rigorous manner. However, considering the number of NTM species and strains that may be implicated in human disease, initial studies may best be focused on MAC and *M. abscessus* strains, because these are the most commonly encountered organisms in NTM PD (2, 3, 23), and *M. abscessus* strains are the most difficult to treat. In parallel, characterization of the species and distribution of NTM within and among patients may give insights into other species and strains that should be prioritized for research, as well as the mechanisms by which certain species or subspecies predominate in specific patient populations. A focused effort to understand pathogen diversity and distribution may identify common virulence characteristics as well as resistance phenotypes. Virulence factors have been identified for MAC (24), including those affecting biofilm formation, and virulence factors for *M. abscessus* have been studied in a number of cellular and animal models (25), Whole-genome sequencing may improve prediction of treatment response, but we must consider complex coinfections with different strains of an NTM species, multiple NTM species, and/or other bacteria and fungi. Within-patient diversity of NTM strains may be significant, and targeted deep-sequencing of patient samples may be necessary to understand population and strain diversity in various anatomical regions of the human lung. *In vitro* susceptibility and clinical response to macrolides and aminoglycosides are linked to genetic resistance markers for both *M. abscessus* and MAC that have the potential to serve as surrogates of phenotypic resistance (26–28). Genome-wide association studies of mycobacteria with antimicrobial susceptibility testing in culture combined with SNP modeling may help further identify resistance conferring mutations.

Bacterial genetics could be characterized to help support molecular testing for diagnostics and possible prognostic precision. Currently, correlation of bacterial genetics and treatment outcomes in patients remains complex. However, before laboratory assays can be established that have the potential to aid in the development of prognostic tools, a more thorough understanding of the mechanisms of virulence in humans must be obtained.

Current laboratory procedures for mycobacteria were almost entirely developed around *Mycobacterium tuberculosis*. Improving recovery and identification of NTM is helped by a new selective mycobacterial medium (RGM) (29) and the increasing use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for mycobacterial identification. Additional simple-to-implement technologies may be required to leverage the diagnostic potential of markers of genetic and phenotypic variation in NTM species, determine antimicrobial susceptibility or resistance, and/or aid in the speciation of NTM in the clinical laboratory. Molecular methods may further contribute to subspecies characterization of NTM, such as a recently developed PCR-based technique for rapid identification of the presence or absence of a full-length *erm*(41) gene (30) in *M. abscessus* group, that could confer macrolide resistance, in addition to known drug resistance markers such as *erm*(41) position 28 C-T. More widespread access to future simpler point-of-care technologies will also facilitate rapid identification of NTM for improved timely treatment.

Development of New Treatments for NTM PD Can Benefit from Advances in TB

The current treatment recommendations for NTM PD vary by species and are

frequently modified by treating physicians (3). On the basis of a limited number of trials and observational studies, culture conversion rates for the recommended MAC regimens are estimated at 65.7% (95% confidence interval, 53.3–77.4%) when drugs were taken for at least 1 year by patients who were macrolide susceptible and had previously untreated MAC (31). In contrast, *M. abscessus* infections and macrolide-resistant MAC are much more difficult to eradicate, and rates of culture conversion remain low (26, 32). Given the significant adverse events during NTM therapy, coupled with low efficacy against certain pathogens, better regimens are urgently needed.

Past drug screening efforts for *M. tuberculosis* found some compounds that were also active against MAC and *M. abscessus* (33). Drug discovery programs should screen separately for *M. abscessus* and MAC, because these organisms are phylogenetically distinct and compounds may be active against only one group. The genetic and phenotypic diversity within *M. abscessus* and MAC requires evaluation against a variety of strains or subspecies at an early step in the drug discovery pathway, as well as when existing antibacterial agents are evaluated for their efficacy against NTM (34). Considering the various microenvironments that NTM occupy in the body, development of NTM-specific *in vitro* potency assays that mimic the state of the pathogen in the host are needed to better correlate *in vitro* susceptibility with clinical efficacy and speed identification of drug candidates (3). Models of slow- or nonreplicating "persister" populations of bacilli, and conditions reflecting intracellular growth, various levels of oxygen tension, nutrient starvation, as well as caseum and mucus, may be important. Pharmacokinetic *in vitro* assays that model "infection-site"–specific drug penetration (for instance penetration into caseum and mucus) have been developed for TB and may have utility for NTM. These assays should be paired with animal model studies that reflect human progressive pulmonary disease. Furthermore, multidrug therapy models are essential, as there is no credible monotherapy approach to NTM PD, all while keeping drug–drug interactions in mind; development efforts focused on regimens rather than single new chemicals would also be advantageous, as has recently been demonstrated for TB (35).

Clinical evaluation of novel drugs and regimens will require standardized case definitions, outcome measures, and comparator regimens, as well as the ability to conduct multicenter trials. Examples from TB (CDC Tuberculosis Trials Consortium) and HIV (NIH/National Institute of Allergy and Infectious Diseases AIDS Clinical Trial Group) clearly demonstrate the importance of clinical trial consortia for providing the necessary expertise, patient population, and data management infrastructure to conduct high-quality trials. Safety and efficacy endpoints, as well as required sample sizes to reach statistical significance, must be aligned with disease characteristics of the patient population to be enrolled. For example, endpoints based on microbiological culture conversion may only be feasible in patients with cavitary disease who present with significant bacterial burden in sputum. Although no consensus has been reached regarding ideal endpoints for efficacy trials in NTM PD, a recent publication did produce consensus definitions of microbiologic and functional endpoints (36), and a recent summary of patient research priorities has highlighted the importance of including quality-of-life outcomes as well (37).

Epidemiologic and Molecular Drivers of NTM Transmission and Disease Need to Be Further Elucidated for Diagnostics and Preventative Interventions

Whole-genome sequencing has recently been used to characterize outbreaks of *M. abscessus* subsp. *massiliense*, showing evidence of possible person-to-person transmission (38). Although the prevalence and geographic extent of transmission remain controversial (39–42), the need for further genetic and epidemiologic characterization of linked isolates is clear. Given the environmental hardiness of these organisms and the potential for fomite transmission (43), epidemiological investigations may provide insight into potential transmission routes in the presence and absence of direct patient contact. Environmental source investigations will require the development of validated processing protocols that are specific to *M. abscessus* and allow sampling of diverse potential environmental reservoirs for this species. Improved recovery of *M. abscessus* isolates will aid outbreak investigations, allow the characterization of the genetic diversity of environmental *M. abscessus* strains, and address whether the globally distributed "clustered" strains of *M. abscessus* are also present in the environment.

Polyclonal infection is likely common, as is reinfection after treatment for MAC and *M. abscessus* (44, 45). Development of biomarkers that differentiate initial, persistent, and subsequent infections will necessitate the establishment of longitudinal patient cohorts to collect well-characterized clinical and microbial specimens and obtain well-curated genetic data. Deep sequencing of microbial genomes from patient samples will also contribute to the characterization of the dynamics of simultaneous and sequential infections with mixed NTM strains and other intercurrent infections.

To Facilitate Focused Biomedical Research in NTM, Enabling Technologies, Models, and Tools Will Have to Be Prioritized

The complexity and diversity of NTM PD, and the difficulty of modeling it in animals, will require new tools. It seems prudent to focus on priority pathogens, for instance *M. abscessus* and MAC. Animal studies may allow the development of initial hypotheses. The analysis of patient databases to conduct genetic and clinical research will facilitate the establishment of best practices that can subsequently be extended to the study of other NTM infections. The "Collaborative Cross" panel of mouse strains reflects extensive host genetic heterogeneity and has promise to advance the field but will have to be directed toward NTM pathogens to be useful (46). Animal models of airway defects similar to those in humans are essential (25). The recent nonhuman primate model may prove useful (47). Bioinformatic tools and patient databases will be required to facilitate international comparison of data. Publicly accessible genetic data from human studies often lack linked clinical metadata, and although CF-specific databases for patients with NTM exist in both the United States (48) and the

United Kingdom (49), as do COPD (50) and bronchiectasis (51) registries, the amount of NTM-specific information captured is variable and is lacking linked genetic data. Harmonization of sequencing approaches and bioinformatic analyses, as well as development of standardized clinical protocols with common data elements and case report forms, will maximize the value of patient data and infrastructure.

Conclusions

Because of its increasing prevalence, difficult diagnosis and treatment, and extremely high recurrence, increased research is needed for NTM PD. MAC and *M. abscessus* require more pathogenesis research and drug discovery. Standardized protocols for whole-genome sequencing analysis and clinical data collection are needed to leverage collaborative research opportunities. Development of vaccines, drugs, and diagnostics will need to be specifically tailored to the hosts and pathogens, as one size will not fit all. ■

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