Moving toward Tuberculosis Elimination

Critical Issues for Research in Diagnostics and Therapeutics for Tuberculosis Infection

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Tuberculosis (TB) has surpassed HIV to become the leading infectious killer of adults globally, causing almost 2 million deaths annually (1). Although this airborne disease has been treatable since 1948, global rates of TB have dropped less than 2% per year; an estimated 10 million incident cases continue to occur annually, including 1 million in children (1, 2). Although transmission of active disease is an important driver of the epidemic, the seedbed that feeds the epidemic is the more than 2 billion people estimated to have TB infection, 5% to 10% of whom will progress to active disease during their lifetime (3). Although any successful strategy aimed at TB

elimination needs to address this reservoir of TB infection worldwide, much remains to be understood about host and pathogen factors that can be used to identify increased risk for progression to disease and intervened on to prevent progression from occurring (4).

The Division of AIDS of the National Institute of Allergy and Infectious Diseases, U.S. NIH, and the Harvard Medical School Center for Global Health Delivery–Dubai convened a group of scientists and stakeholders on September 28 and 29, 2017, to address knowledge gaps that affect our ability to rapidly find and treat individuals infected with *Mycobacterium tuberculosis* who are most likely to progress to active

disease. The meeting identified a number of efforts underway to address this important gap in the collective ability to stop the global TB epidemic. Here, we review and outline the priority areas for research, diagnosis, and treatment of TB infection that emerged from the meeting (Table 1), building on recent reviews in this area (5–7).

Understanding the Spectrum of TB Infection and Disease: A Critical First Step

Although epidemiological data from the late 1950s—and a number of randomized

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Table 1. Key Research Areas and Questions in Diagnostics and Therapeutics

1. Defining the spectrum between *Mycobacterium tuberculosis* infection and clinically apparent disease and identifying genetic and immunological markers associated with progression to disease
 - Where in the host is the *M. tuberculosis* population during TB infection, are there niche tissues, and do different locations and environments affect bacterial behavior, host response, and treatment response?
 - What is the physiologic and metabolic state of the population of *M. tuberculosis* along the spectrum between infection and clinically evident disease, and could this be used to target individuals for treatment?
 - Are there host and pathogen genetic markers (“gene signatures”) that define these states, and what is the interplay between the two?
 - Are there host immunological/cellular function responses that define these states?
 - Are there protein biomarkers that define these states?
 - Can any markers be identified that indicate the presence of live *M. tuberculosis*?
 - Can markers of bacterial clearance in response to chemotherapy or therapeutic vaccines be identified?
 - Are genetic, immunological, and protein biomarkers the same between adults and children, and how can differences between age groups be used to better understand the spectrum between TB infection and disease?
 - How is progression along the spectrum to disease affected by host and pathogen genetic diversity?
 - What are the correlates of protection from TB disease?
 - How do the above processes differ between young children and older children/adults, and what bearing will this have on identification of infection in this population?
2. Optimizing treatment for infection with *M. tuberculosis*
 - Which drugs and regimens are optimal for treatment of incipient forms of TB?
 - What are the drug mechanisms of action that could safely shorten duration of treatment for TB infection to 4 or fewer weeks?
 - Is there a role for host-directed and adjunctive therapy to modulate inflammation, lipid metabolism, autophagy, and other cellular networks to disrupt the transition between TB infection and disease?
 - How can host-directed and adjunctive therapies be used to reduce the duration of treatment for TB infection?
 - What anti-TB drugs are amenable to long-acting/extended-release formulations that will help maintain more constant antibiotic coverage and help with treatment adherence?
 - What anti-TB drugs are amenable to alternative treatment delivery mechanisms (e.g., drug patches, sustained release injectable depot drug delivery)?
 - What drugs should we use to treat individuals suspected of being infected with multidrug-resistant strains of TB?
 - What molecular markers, immunological responses, and protein biomarkers can help define when infection and/or disease has been successfully treated?
 - Do children and adults require the same treatments and durations for TB infection?

Definition of abbreviation: TB = tuberculosis.

controlled trials since then—demonstrate the importance of treating TB infection before it becomes clinically apparent (preventive therapy for so-called “latent” TB) (8–12), very little is known about the spectrum of clinical states between asymptomatic infection and clinically apparent disease. It is becoming increasingly clear that conventional conceptions of TB as “latent” versus “active” are overly simplistic and adequately reflect neither host immunology nor microbial pathogenesis (13–15). Indeed, there is now increased appreciation that clinically apparent “TB disease” is assuredly preceded by a period of incipient TB—an asymptomatic phase that can be

characterized by an evolving blood-based host RNA signature (16). These observations confirm that the previous classification of “latent” infection is imperfect and limited by the characteristics of available diagnostic tests (17). Moreover, it is hypothesized that some exposed individuals can mount effective immune responses that eradicate all viable bacilli, a phenotype distinct from persons in whom immune responses can only contain the infection, thereby harboring populations of bacilli that intermittently replicate in macrophages, granulomata, and other tissues (4). Whether the newer terminology of “incipient” disease indicates early active TB that inevitably progresses to clinically

apparent disease, or whether it is more of a dynamic state that reflects ongoing host–pathogen interactions, remains unknown. In addition, the definitions and pathophysiology of a preincipient state is also unknown. Being able to characterize and identify the full spectrum of “latency,” including the dynamics of microbial and host interplay and the role this relationship has in developing disease, is a critical component to addressing the TB epidemic (Figure 1).

It has long been hypothesized that subclinical TB infection is characterized by a population of *M. tuberculosis* in a dormant or metabolically quiescent state (18, 19). Rather, recent data on changes in host immunity during TB infection and the efficacy of treatment for TB infection point toward a population of organisms that have substantive metabolic activity (11, 20). The true nature of bacterial metabolism, and associated vulnerability to antitubercular drugs, remains a critical knowledge gap that has been difficult to study in humans because of the inability to meaningfully sample bacteria before TB disease progression (16). To address this, *in vitro*, *ex vivo*, and *in vivo* models have been established to study various aspects of nonreplicating persistence in mycobacteria. Passage of *M. tuberculosis* through these models reveals a dynamic and complex pathogen response to the immune stresses imposed during infection (16). For example, *in vitro* models that simulate the nonreplicative state demonstrate that carbon metabolism is extensively remodeled to accommodate a reduction in simple glycolytic substrates and an increase in the availability of fatty acids and host-derived cholesterol (21–24). Similarly, nitrogen metabolism is rewired to respond to increasing availability of nitrate, a byproduct of host-derived reactive nitrogen stress (25, 26). *In vitro* models and infection studies in animals show specific pathogen metabolic adaptations that address the reduced levels of oxygen present in the TB granuloma (16,27–29). Collectively, data from these models of nonreplicating mycobacterial persistence suggest that the bacteria are not metabolically quiescent. Although this finding may provide an explanation for the effectiveness of preventative therapy with agents that target active cellular processes given over an extended time frame (e.g., isoniazid [INH] and rifampin) (4–9), it is not yet clear how

Figure 1. Spectrum of clinical states that comprise asymptomatic tuberculosis (TB) infection. Shown are the various manifestations of infection with *Mycobacterium tuberculosis*, before the diagnosis of active TB. The spectrum of TB-infected states, which were previously all characterized as latent TB infection, represents a diversity of outcomes on exposure to infectious particles. This appreciation of clinical complexity has been largely driven by the development of biomarkers to monitor risk of disease progression and refined imaging techniques. Text below the graphic details areas of investigation necessary to better characterize the latency spectrum, including study of bacterial physiology, novel diagnostics, HDTs, new therapeutic options, and host immunity. The ultimate goal of further understanding the spectrum of TB infection is to develop interventions that prevent progression to active TB disease. H = isoniazid; HDT = host-directed therapies; IGRA = IFN- γ release assay; LTA4H = leukotriene A(4) hydrolase; P = rifapentine; R = rifampin; TLR2 = Toll-like receptor 2; TST = tuberculin skin test.

much these *in vitro* models correspond to mycobacterial activity in humans.

There are many unanswered questions regarding the ability of tubercle bacteria to survive in an immune-active host. Gaps exist in understanding where bacteria are located during the early stages of TB infection and whether different locations and environments affect bacterial behavior and treatment response (11). How similar is the bacterial metabolism along the spectrum between infection and disease? If there are differences, how do we define vulnerable drug targets for TB infection? Would recognition of bacterial behavior during early conditions allow us to define new growth/media conditions for diagnosis? These are all critical areas of inquiry.

The interplay between bacterial and host genetics and the subsequent combinatorial effect on host immune response during infection is another important area of research. First, we know that *M. tuberculosis* can interact with a large repertoire of innate host receptors that shape the immune response and outcome of infection and progression to disease (30). For example, eicosanoid generation, considered as a host-protective response to mycobacteria, is regulated by leukotriene A(4) hydrolase (LTA4H). In tuberculous meningitis, a genetic polymorphism in LTA4H is associated with both inflammation and responsiveness to adjunctive antiinflammatory therapies (31). Second, immune responses appear to also be influenced by *M. tuberculosis* strain diversity. *M. tuberculosis* complex comprises seven human adapted lineages, and studies have reported that genotypic diversity is associated with differences in host immune responses (32–34). That the interaction between host polymorphisms and TB strain variation may dictate the outcome of infection is illustrated in a study that showed that the association of SNP TLR2 (Toll-like receptor 2) allele T597C with risk of TB meningitis is strongest among those infected with the Beijing lineage (35).

Overall, it is plausible that the spectrum of infection is influenced by a combinatorial effect of both host and bacterial genotypes. A better understanding of the nuances of host–pathogen dynamics during different *M. tuberculosis* infection states and with *M. tuberculosis* genetic diversity would contribute to developing protective vaccines

for TB infection and therapeutic vaccines for treatment. It would also help to identify biomarkers of pathogen clearance and of progression to TB and advance our understanding of host innate and adaptive immunity to *M. tuberculosis*. This is critical for eradicating persistent infection, preventing reactivation of TB infection, shortening TB infection treatment, and/or preventing reinfection.

Diagnosing TB Infection and Progression to Disease

Another major gap in addressing TB infection has been the inability to differentiate between asymptomatic individuals harboring viable persistent bacteria versus those who have cleared the infection. This is highly relevant for the accurate identification of infected individuals who are at greatest risk for progressing to TB disease. Although persons with positive IFN- γ release assays specific for TB antigens are at increased risk of progressing to clinically apparent disease, the number needed to treat to prevent a single active case can range as high as from 111 to 314 (depending on setting and the patient's risk for progression), an observation that has dampened enthusiasm for universal treatment of TB infection (36). Although recent studies have identified host biomarkers and transcriptional profiles associated with active TB and signatures associated with TB disease risk (37–39), signatures that distinguish persistent TB infection from cleared infection have not been identified. Moreover, the effect on these signatures by coinfection with HIV or helminths, or comorbidities such as diabetes, is not known.

Overall, there are substantial problems with current definitions of infection, because they are generally inferred from surrogate assays that measure the metabolic, transcriptomic, or bimodal immunologic response of the host to an encounter with *M. tuberculosis*. The extent to which surrogate markers reflect characteristics of infection—versus “conversion”—remains unknown. It is recognized that individuals postconversion exhibit both increased risk for progression and heterogeneity in timing of progression. Exposed individuals who do not convert may represent a “resistance to infection” phenotype. Genetic mapping efforts of *M. tuberculosis* infection offer particularly powerful approaches

for identifying loci that confer resistance to infection or that control progression from conversion to clinical disease. These efforts may also be useful in fine-tuning surrogate markers to improve the accuracy of prediction of progression to clinical disease. For example, transcriptomic signatures have been identified that provide a correlate of risk for progression from conversion to disease with relatively good accuracy (40). By including genetic markers that impact transcript levels, so-called expression quantitative trait loci, it may be possible to further improve the accuracy of these signatures. Much needs to be done in this area.

Despite the checkered history of serologic assays in TB, more refined understanding of the antigen binding (Fab) and constant (Fc) domains would support accurate antibody-based diagnostics. Most antibody work has focused on the Fab domain, given the specificity of association with microbial antigens. Unfortunately, this approach has thus far given rise to commercial serological tests with poor sensitivity and specificity for TB infection and disease. However, it is well understood that immunogenic microbial antigens are not just proteins but also lipids and carbohydrates. Recent studies have shown that the “constant” Fc domain is actually diverse, capturing a plethora of host-associated processes (41). More specifically, isotypes ($n = 5$), subclasses ($n = 6$), and post-translational modifications such as glycosylation ($n =$ at least 36) are dynamic throughout the course of disease, reflecting the host immune responses (42). These Fc features, specifically glycosylation, have been successfully used in oncology to functionally enhance monoclonal antibody therapeutics, in rheumatology to provide more predictive and granular biomarkers, and in HIV diagnostics (43–45). Overall, these important advances in our understanding of the role of antibodies suggest that further research is essential (46).

The measurement of other aspects of cellular immunity to mycobacterial infection may provide signals that discern TB infection from active disease and hence might be used to define those with subclinical TB. As *M. tuberculosis* is an intracellular pathogen, CD8⁺ T cells are uniquely poised to sample the intracellular environment. The prevalence of *M. tuberculosis*-specific CD8⁺ T cells is associated with surrogates of bacterial burden, such as sputum-smear positivity,

and is inversely correlated with treatment. Innate T cells, such as invariant natural killer cells and mucosal-associated invariant T cells, are diminished in the circulation of individuals with *M. tuberculosis*, possibly reflecting their movement to the site of active infection. Increased numbers of myeloid-derived suppressor cells in blood of recently exposed individuals and in TB and increased numbers at the site of disease in pleural TB have been found, whereas these numbers normalize after prolonged asymptomatic infection and after successful TB treatment. These cells suppress innate and adaptive immune responses against TB (47, 48). Expression of immune activation markers, such as CD38 and HLA-DR, and intracellular proliferation markers, such as Ki-67, on *M. tuberculosis*-specific CD4⁺ T cells have been shown to accurately discriminate between active TB disease and TB infection, suggesting that antigen-specific T-cell phenotypes can be useful as biomarkers of TB disease (37). Finally, the measurement of the functional status of *M. tuberculosis*-specific CD4⁺ T cells—using either IL-2 production or cellular activation—has been demonstrated to discern TB from TB infection (49). In summary, understanding better the host response to infection with *M. tuberculosis* has the potential to predict those at risk for progression, add specificity to RNA signatures, and guide therapy.

As high TB incidence areas are often characterized by high sensitization rates to *M. tuberculosis* antigens (i.e., tuberculin skin test or IFN- γ release assay positivity), these tests are of limited value in selecting healthy individuals for targeted preventative treatment (50). Recent publications have highlighted a new approach using gene signatures to predict future progression to clinically active disease in people who are apparently healthy at the time of testing (37, 51, 52). The Adolescent Cohort Study reported the generation of a risk signature from whole-blood RNA sequencing followed by validation through multiplex quantitative real-time PCR in adolescents without a known exposure episode from a high TB-incidence community. The expression of 16 mRNA transcripts constituted a correlate of risk (CoR) for active TB disease and was validated in a cohort of recent household contacts of active TB cases from South Africa and The Gambia (the GC6-74 study) (35). The CoR progression in the adolescent cohort was similar to CoR

regression toward a control state in an independent TB treatment cohort that was recently reported from South Africa (53). Subsequent further analysis of the GC6-74 cohort showed similar promising predictive performance across household contacts from multiple sites, suggesting that predictive signatures are indicative of incipient disease up to 2 years before the development of symptoms and that signatures that perform reasonably well across different geographical areas should best be developed from data sets that include samples from multiple locations (39). Such signatures are now being tested prospectively in clinical trials of biomarker-driven targeted preventative treatment (37).

Newer discovery platforms, like next-generation biological mass spectrometry, coupled with methods for enhancing sensitivity, such as exosome enrichment, are being evaluated for the detection of pathogen-specific markers across the spectrum of TB infection to disease (54, 55). Pathogen-specific diagnostics would provide a valuable alternative to surrogate marker approaches and be able to discern true “infection” in *M. tuberculosis*-exposed patients from immunologic “conversion” after exposure.

Last, the fundamental challenge of how to optimize the evaluation of diagnostics for TB infection, for which there is no gold standard, needs to be addressed. Historically, active TB disease cohorts have been used, but the severe immunologic and pathogen features of this state are recognized as poorly reflective of the predisease, infection state. Proxies for infection (e.g., on the basis of tuberculin skin test or IFN- γ release assay results) are also used in studies, including proposed new cut-offs for defining positive results (56), and these can be further enhanced by determining whether a novel biomarker or diagnostic test separates the study population into two groups of size consistent with the known epidemiology of TB infection: the majority (80–95%) with stable TB infection and a minority (5–20%) identified as being at risk (i.e., unstable TB infection or subclinical TB). This approach still has risk in terms of misclassification, but candidate biomarkers that achieve this goal could then be advanced to phase 2 studies that assess changes in the candidate biomarker levels with treatment of TB infection. In the absence of a reference standard, assessing for treatment response is also a reasonable approach and is supported by prior studies demonstrating

that gene expression is similar among patients who have completed treatment for TB disease and control subjects with TB infection (57). Such phase 1 and 2 studies require smaller sample sizes and shorter follow-up as compared with large epidemiologic cohorts and could help identify potential candidate biomarkers or tests to advance to clinical impact studies that assess TB incidence.

Innovations in Treatment of Infection

Recently completed trials of duration-shortening regimens for drug-susceptible TB infection have confirmed the importance of treating TB infection and provided viable alternatives to the conventional 9 months of INH (9H) used since the 1960s (5–9). Several randomized controlled trials have shown that a new combination regimen of INH and rifapentine (RPT) administered weekly for 12 weeks as directly observed therapy or as self-administered therapy is as effective for preventing TB as other regimens and is more likely to be completed than the longer 9 months of INH daily (58–60). A 4-month regimen of rifampicin has been evaluated in adults and children with TB infection (NCT00931736) and found not to be inferior to the standard 9-month regimen of INH for the prevention of active tuberculosis and, notably, is associated with a higher rate of treatment completion and better safety (61). Two ongoing studies are evaluating the effectiveness of preventive therapy among people living with HIV: a 1-month course of daily RPT and INH versus 9H (the A5279 study; NCT01404312) was recently completed, and preliminary results indicate noninferiority of the ultrashort regimen when applied under pragmatic strategy trial settings (62); and a three-arm comparison between 3 months of isoniazid plus rifapentine once weekly (3HP), 9H, and intermittent 3HP is being evaluated among people living with HIV (the WHIP3TB study; NCT02980016). The durability of protection achieved from a single 6-month course of INH treatment for TB infection in people living with HIV remains uncertain, with some trials showing up to 7 years of protection (63), whereas others found significant benefits from prolonged courses (36 mo) of INH treatment in high-incidence settings as compared with the 6-month regimen (64). The CORTIS study

is evaluating the effectiveness of 3HP for high-risk individuals, against surveillance, with the treatment decision based on a blood-based RNA-expression profile (NCT02735590). Supervised weekly 3HP is being compared with INH among pregnant and postpartum women (P2001; NCT02651259). Finally, 6 weeks of daily, self-administered RPT-alone regimen is being evaluated in a new phase 3 clinical trial conducted by the CDC TB Trials Consortium (65).

Currently, there are limited data on how best to treat contacts of individuals with multidrug-resistant (MDR) and extensively drug-resistant TB. Several drugs or combinations of drugs, including fluoroquinolones, ethambutol, pyrazinamide, and ethionamide, have been tested in modest-sized trials that have not provided adequate evidence to impact practice guidelines. Currently, three large, randomized controlled trials are underway. Six months of daily levofloxacin is being compared with placebo for contacts of individuals with MDR-TB in the VQUIN MDR Trial (ACTRN12616000215426, in adults) and the TB CHAMP study (ISRCTN92634082, in children younger than 5 yr of age). In the PHOENIX MDR-TB Trial, 6 months of daily delamanid will be compared with 6 months of daily INH for contacts of individuals with MDR- or extensively drug-resistant TB.

Current treatment studies focus on the use of orally administered antibiotics for which susceptibility is presumed; however, there is interest in evaluating novel, nonantibiotic-based approaches to treating infection and halting progression to disease, as well as alternative drug-delivery mechanisms. The idea of using host-directed therapies as adjunctive therapies for TB has gained traction (66). Theoretically, host-directed therapies can address the challenges associated with antibiotic resistance, as bacterial mutations will be incapable of directly abrogating binding of the drug to a host target. In addition, these therapies open a new landscape of ways to harness or alter host immunity to attack the pathogen. A number of host-directed therapies have been proposed based on modulation of detrimental inflammation—more classically considered in the context of clinically apparent disease—including alterations of eicosanoid networks, lipid metabolism, and autophagy, among others (67–69). Questions remain about whether

these newer host-directed approaches would be effective for addressing the spectrum of infection, where the inflammatory response may be different from active disease. Answering these questions is an important first step in determining the role of adjunctive host-directed medications or therapeutic vaccines in the prevention of progression from infection to disease (5).

There remain a number of challenges in efficiently transforming preclinical results and clinical observations into specific adjunctive therapies, particularly given the heterogeneity in disease presentation and progression. Cell culture, animal models, human clinical data, and long-term experiments in nonhuman primates (e.g., cynomolgus macaques or rhesus macaques, where infection with *M. tuberculosis* results in similar outcomes as the human infection/disease spectrum) are needed to capture the full spectrum of possibilities with adjunctive therapies. Ultimately, novel adjunctive therapies will require initial assessment of safety, consideration of potential interactions with front-line anti-TB drugs, and knowledge of efficacy in preclinical models.

Ensuring that treatment can be delivered is also a critical area of inquiry. Long-acting/extended-release drug formulations have proved very successful in diverse areas of medicine including contraception, psychiatry, and, most recently, HIV disease. Although challenging, application of this technology to TB treatment could have substantial impact by improving treatment adherence. Although the properties of some TB drugs make them unsuitable for long-acting formulation, promising candidates have been identified through modeling and simulations (70–73). An efficacious delivery mechanism for TB infection, particularly for those coinfecting with HIV, would be an important tool to accelerate progress toward TB elimination. Progress in this area is closely linked to the identification of markers associated with disease clearance and progression.

Understanding host–pathogen interactions in the setting of high transmission pressure is also an important facet of combatting the disease. Although many of the early studies on preventive therapy were undertaken in high-burden settings (5–9), and models of disease elimination have underscored the importance of using a comprehensive epidemic control approach strategy that

includes active case finding, the treatment of active disease, and the treatment of infected contacts (74–76), research is required to better understand how targeted identification of infected individuals in “hotspots” and other epidemiological contexts could advance the goal of TB elimination (77).

Last, it must be remembered that most of the studies on the relationship between *M. tuberculosis* and its human host have been conducted in adults. There are an estimated 10 million new pediatric TB infections each year (78). Once infected with *M. tuberculosis*, the risk of disease progression is much higher in children, especially those younger than 5 years of age (79). Because of differences in host immunity between young children and older children and adults, this population faces a different series of gaps in diagnostics and therapeutics. Studying TB infection in children—who usually have a defined contact and hence defined time since exposure—is a unique “challenge model” to examine time- and possibly strain-related infection progression and protection (80).

Conclusions

TB elimination can only be achieved if the reservoir of infected individuals at high risk of progression to disease are identified and treated. Recent advances in our understanding of host–pathogen interactions in TB, coupled with newer approaches for diagnosis, predictive biomarkers of risk for progression, and the promise of shorter-duration treatments, adjunctive therapies, and innovative modalities for drug delivery are providing a new horizon for research. For the first time, the collective of these advances provides an exciting and viable platform on which to build the next generation of interventions for identifying and treating TB infection. ■

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