SUPPLEMENT ARTICLE

Viewpoint: Challenges and Opportunities in Tuberculosis Research

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Partially fueled by the human immunodeficiency virus (HIV) pandemic, tuberculosis is the second-leading cause of infectious diseases–associated mortality globally and the leading cause of death among those infected with HIV. In 2010, there were an estimated 8.8 million incident cases of tuberculosis and approximately 1.5 million deaths attributed to tuberculosis. Increased public heath efforts have been effective in decreasing the overall prevalence and mortality associated with this disease, but declines in incidence rates have been outpaced by global population growth, such that absolute incidence rates have not declined as fast as previously expected [1]. Commensurate with increased public recognition of the importance of tuberculosis to global health in the mid-1990s, biomedical research for tuberculosis has increased both in the United States and internationally. Although tools to conduct modern biomedical research involving tuberculosis have only been available for a little over a decade, this research has resulted in several successes, including promising new vaccines to protect against tuberculosis in the highest-risk populations, diagnostic candidates to improve the accuracy and speed of diagnosing all forms of tuberculosis, and new drug candidates with the potential for improved treatment and chemoprevention for drug-sensitive and drug-resistant tuberculosis.

Despite these successes, many questions remain in all aspects of tuberculosis research, ranging from fundamental pathogenesis to programmatic implementation, and they are complicated by the complexity of the disease and its chronic nature. Compared with other infections, *Mycobacterium tuberculosis* infection does not elicit clinically relevant disease in the majority of subjects, and many who are infected carry asymptomatic, paucibacillary disease for decades. This complicates the definition and evaluation of early infection events and initial host responses and the characterization of infecting bacillary populations. The clinical definition of latent infection is limited to positive responses to injection of purified mycobacterial components (ie, the tuberculosis skin test) or to T-cell–recall responses measured through the production of interferon γ; unlike viral infections, latent tuberculous infections cannot be identified on the basis of stable antibody titers. Progression to active disease is not a clear unidirectional process and is prolonged, and many affected persons appear to be able to clear the infection, whereas others progress to active disease. The spectrum of active tuberculosis is complex. Although adult pulmonary tuberculosis is considered of greatest relevance for public health, extrapulmonary disease occurs in a significant portion of subjects, and pediatric manifestations of tuberculosis may be mistaken for other pulmonary infections and are difficult to diagnose. Treatment of active disease is complicated by the fact that the current standard of care involves combinations of drugs whose administration is sequenced on the basis of the clearance of mycobacteria from sputum and that, unlike most other antibacterial treatments, have to be administered for a minimum of 6 months to elicit a stable cure. Hypotheses exist that explain the need for such prolonged therapy, but they are difficult to test experimentally because initial treatment of tuberculosis eliminates bacteria from sputum and thus limits options for studying bacterial

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populations. Unlike many other infectious diseases, in tuberculosis, patients do not become immune to reinfection at a later time. Thus, a clear definition of the markers of immune protection is dependent on successful trials of new vaccines or has to be derived from data for persons who were infected but did not progress to active disease. However, both criteria are complicated by the limitations listed above.

Comprehensive reviews of research priorities that have been defined on the basis of the unique nature of tuberculosis and the associated scientific/technical limitations of studying the disease have been addressed elsewhere [2–5], but several issues merit further exploration.

FUNDAMENTAL RESEARCH

An understanding of the differences and commonalities of tuberculosis among high-burden countries is critical for a better understanding of the global tuberculosis epidemic, the natural history of the disease, and, ultimately, the development of appropriate scientific approaches for discovery and development of drugs, vaccines, and diagnostics applicable in these settings (Ibrahim Abubakar, et al, ''Drug resistant tuberculosis—current dilemmas and unanswered questions,'' this *Journal of Infectious Diseases* supplement). In particular, the evaluation of the natural history of tuberculosis in the context of coinfections, comorbidities, risk behaviors, and other factors that are prevalent in these countries is critical to understanding tuberculosis in its natural setting. The complex interplay between pathogen and host immunity and the contribution of strain genetics to host immune response, transmission, pathogenicity, and acquisition of resistance need to be better understood to develop interventions that are either targeted to a specific setting or applicable worldwide (Susanna Brighenti and Jan Andersson, ''Local immune responses in human tuberculosis: learning from the site of infection, this *Journal* supplement; Rebecca Axelsson-Robertson et al, ''The immunological footprint of *M. tuberculosis* T-cell epitope recognition,'' this *Journal* supplement). In particular, our understanding of transmission and of the nature and/or number of sequential exposure events required for infection and eventual latency or active disease are limited and complicate our characterization of the associated early host and pathogen factors. Application of state-of-the-art genomics, systems biology, and creative bioinformatics analyses promise to shed light on the complexity of these interactions. With further development and refinement, molecular investigations of the genetic factors underlying virulence, pathogenicity, and drug resistance in *M. tuberculosis* may provide the backbone for the design of drugs, vaccines, and diagnostics.

Epidemiologic data are essential to properly inform the development of implementation research agendas to ensure that investments in product development are properly paired with in-country science, to facilitate clinical trials and product roll out (Frank Cobelens, et al, ''Which new diagnostics for tuberculosis, and when?'' this *Journal* supplement). Although implementation research has traditionally been regarded separately from basic and clinical research, the HIV and tuberculosis epidemics exemplify the importance of addressing the ultimate goal—to use new research developments to deliver better care and end both epidemics. Prescient consideration of operational issues promises faster translation of scientific discoveries and new technologies into clinical use. Optimally, clinical development and implementation research are done in parallel to decrease delay in programmatic implementation. For example, implementation research done now to translate current childhood immunization programs into effective programs for adolescents and adults will be critical to the rapid roll out of licensed tuberculosis and HIV vaccines in the future. The recognition that fundamental research is a critical component of the global fight against tuberculosis and the prerequisite for development of new healthcare interventions for tuberculosis has recently been highlighted by inclusion, for the first time, of a chapter on research and development in the World Health Organization's annual report on global tuberculosis control [1] and by summarization of these activities in the research roadmap of the Stop TB Partnership's TB Research Movement [5].

PREVENTION

An effective vaccine may offer the best hope to eventually eradicate tuberculosis. The only licensed tuberculosis vaccine, BCG, is the most widely used vaccine globally. More than 100 million doses of BCG vaccine are given annually to protect infants in high-burden countries from tuberculosis, including often fatal forms of extrapulmonary tuberculosis. The BCG vaccine's efficacy in protecting against adult pulmonary disease greatly varies globally, and it is not considered a reliable tool for tuberculosis control. The current pipeline for tuberculosis vaccines includes a diverse collection of delivery platforms and adjuvants, live attenuated vaccines, and prime-boost combinations [6, 7].

Despite increased efforts in tuberculosis vaccine research, large gaps exist in our understanding of tuberculosis. Tuberculous mycobacteria are able to evade host immune responses, and factors associated with establishment of immune memory after infection/disease are unclear (Rebecca Axelsson-Robertson et al, ''The immunological footprint of *M. tuberculosis* T-cell epitope recognition,'' this *Journal* supplement). This, coupled with the prolonged time between infection and disease manifestation and priming of cross-reactive immune responses by exposure to environmental mycobacteria and/or BCG vaccine, complicates assessment of the status of tuberculous infection.

Vaccination approaches are therefore not limited to primary prevention of infection, but include vaccines to prevent disease activation and therapeutic vaccines as adjuncts to chemopreventive therapy for persons already infected.

Additionally, recent evidence [8] suggesting the efficacy of short-duration, combination, chemoprevention strategies is an important development that deserves further consideration, especially in HIV-infected persons and other immunocompromised populations. In addition to evaluation of new combinations/approaches to further increase efficacy and shorten required treatment duration, implementation research on the feasibility of chemoprevention strategies and their potential impact on treatment programs and local tuberculosis epidemiology are also needed. Furthermore, treatment of individuals (both adults and children) exposed to drug-resistant tuberculosis represents an important knowledge gap that requires evaluation.

The current tuberculosis prevention portfolio is complex and diverse. To address the absence of surrogate markers of efficacy, immune-response assays are being developed as vaccines are tested in humans. Current clinical trials offer an opportunity to search for useful correlates of protection. Additionally, the availability of better epidemiologic data would help inform trial design in the setting of changing demographic data, increasing coverage with antiretroviral therapy, and aging among the populations at highest risk.

TUBERCULOSIS DIAGNOSIS

Improving the diagnosis of latent tuberculosis infection, identifying persons most likely to advance to active disease, rapidly detecting pulmonary and nonpulmonary tuberculosis by use of nonsputum samples, identifying persons most likely to relapse after treatment, and developing affordable, rapid methods for identifying drug resistance are critically needed advances. These advances will require a detailed understanding of the dynamics and molecular/immunologic characteristics of the disease in humans and the identification of biomarkers (host and microbial) indicative of the various stages of disease and treatment response. The complexities involved in this research portend that significant advances will not be easy, but the potential impact of such biomarkers is compelling. Besides the obvious clinical usefulness (eg, allowing prioritization and/or improved prediction of individuals requiring chemopreventive treatment), these tests could be used as surrogate end points to improve the efficiency of early phase evaluations and to greatly reduce the prolonged follow-up period currently required for late-phase drug and vaccine clinical trials [9, 10].

Nucleic acid amplification technologies coupled with molecular detection systems represent a very important modern achievement in the field of tuberculosis diagnostics. Line probe assays and the Xpert MTB/RIF test provide laboratories with the ability to accurately diagnose tuberculosis and detect multidrug-resistant tuberculosis in a fraction of the time required by traditional culture-based methods. The <2-hour diagnosis time afforded by the Xpert MTB/RIF assay for detection of both tuberculous disease and rifampin resistance has the potential to significantly impact the care of patients with tuberculosis and, particularly, those with drug-resistant tuberculosis, provided the technology continues to be made available to laboratories in high-burden countries (Ruth McNerney et al, ''Tuberculosis diagnostics and biomarkers,'' this *Journal* supplement). Despite these successes, effective tuberculosis diagnosis and detection of drug resistance still remain elusive in many regions of the world, especially in pediatric and HIV-infected populations. Research is needed to determine whether these assays will also be effective for accurate diagnosis of extrapulmonary tuberculosis and childhood tuberculosis (Stephen Graham et al, ''Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical definitions,'' this *Journal* supplement; Luis Cuevas et al, ''Evaluation of tuberculosis diagnostics in children: 2. Methodological issues,'' this *Journal* supplement). In addition to the need to strategically ramp up implementation of new assays, true point-of-care diagnostics that can quickly and accurately diagnose tuberculosis without electricity and technical expertise at low costs are needed for first-line clinics in resource-limited settings (M. L. Schito et al, ''Opportunities and challenges for cost-effective implementation of new point of care diagnostics for HIV and Tuberculosis,'' this *Journal* supplement). Although much work is ongoing, combination strategies (multianalyte and multiplatform) may be required to overcome the weaknesses of individual diagnostics and to efficiently address detection of coinfections, such as HIV infection and hepatitis B and C virus infections.

Closely tied to the development of new combination therapies for tuberculosis is the need to rapidly and accurately detect drug resistance at the patient level and catalog existing drug resistance patterns at the population level. While the clinical definition of multidrug-resistant and extensively drug-resistant tuberculosis covers resistance to isoniazid, rifampin, fluoroquinolones, and the injectable drugs, drug resistance testing for other first- and second-line agents, against which clinical resistance may be significant, is not commonly included in national surveys. For pyrazinamide, difficulties associated with culture-based assessment of resistance present challenges for patient care, public health monitoring, and drug development. As new drugs are made available for general use, strategies to prevent rapid development of resistance to additional drugs in effective multidrug regimens will be important. Assays for molecular

detection of drug resistance have the potential to meet this need [11]; however, data are needed on the clinical usefulness of genotypic resistance profiles to determine optimal drug regimens for clinical care. Further determination of the full spectrum of clinically relevant drug resistance mutations is also critical (P. Nahid et al, ''Clinical research and development of tuberculosis diagnostics,'' *Journal* supplement, this issue).

Besides diagnostic platforms intended for clinical care, tests with specific usefulness for drug-development studies are also needed. Studies correlating high-resolution computed tomography (CT) and positron emission tomography (PET)/CT to treatment outcomes indicate that imaging modalities may have the potential to serve as early surrogate biomarkers in clinical drug combination development (L. Via, personal communication). Development of improved tracers with longer half-lives and better specificity for tuberculous mycobacteria is being pursued, and magnetic resonance imaging/PET may provide further advances. Standardized imaging protocols, data elements, analysis methods, and optimal cutoff criteria to define the degree of treatment response will need to be developed.

Development of a real-time quantitative ribosomal RNA assay for sputum analysis also appears to be a promising technique for measurement of viable bacteria in serial sputum samples, including nonreplicating persistent organisms [12]. Its application to phase II studies could replace current culture-based methods and could be more cost effective. Real-time measurements like this assay and imaging technologies will greatly facilitate the efficiency of clinical drug development research by facilitating rapid decision making in phase II adaptive trials (P. Phillips et al, ''Innovative trial designs are practical solutions for improving the treatment of tuberculosis,'' *Journal* supplement, this issue).

TUBERCULOSIS TREATMENT

New treatment regimens for patients with drug-resistant and drug-sensitive tuberculosis are urgently needed. The long duration and poor tolerability of current regimens represent important challenges to curbing the tuberculosis pandemic. The global incidence of multidrug-resistant tuberculosis currently stands at 3.6% of all tuberculosis cases, with substantially higher rates in certain regions such as eastern Europe, southern Africa, and central Asia, and in cases of tuberculosis retreatment as compared with new cases [13]. The high rate of default and treatment failure associated with current tuberculosis regimens under programmatic conditions is a contributing factor to this problem, and new, more effective, and shorter treatment regimens and other treatment improvement approaches for both drug-sensitive and drug-resistant tuberculosis are essential.

With a pipeline of new drugs for tuberculosis that is more robust than ever, the efficient evaluation of individual agents and combinations will be critical. Advanced planning and coordinated assessment of treatment combinations is a high priority to ensure that development of new drug regimens is based on pharmacological and antimicrobial compatibility and results in the most efficacious and safe new regimens. While early-phase studies may give a relatively rapid and early indication of optimal dose and drug combinations, the use of disease relapse as the ultimate end point for clinical efficacy will require large and prolonged phase III trials to confirm long-term safety and efficacy. Development of regimens on the basis of the most appropriate preclinical and animal model tests and use of adaptive trial designs (P. Phillips et al, ''Innovative trial designs are practical solutions for improving the treatment of tuberculosis,'' *Journal* supplement, this issue) will facilitate optimal use of limited clinical trials capacity and provide opportunities for the addition of nested studies of biomarkers to establish their use for prediction of clinical treatment response.

Rational design of optimal multidrug regimens will require consideration of multiple factors beyond clinical efficacy, including drug interaction effects, pharmacokinetics, toxicity, drug metabolism, and the thresholds for development of resistance associated with the component drugs. Antituberculous drugs with high barriers to resistance will be important not only for their therapeutic efficacy, but also for their ability to protect other drugs in the combination regimen. Sensible drug sequencing/staging strategies to improve outcomes with current drugs are also needed. These strategies would capitalize on known mechanisms of action and activities against the spectrum of bacterial subpopulations (from rapid replicators to semidormant organisms) and minimize antagonistic interactions (eg, between isoniazid and pyrazinamide). Additionally, with the recognition that patients with tuberculosis frequently present with coinfections and comorbidities, compatibility of antituberculous drugs with drugs to treat comorbid conditions such as heart disease, diabetes, HIV infection, and other chronic viral infections must be assessed at the earliest possible stage in clinical development. Comparative studies are needed because some current regimens, particularly those to treat multidrug-resistant and extensively drug-resistant tuberculosis, are poorly characterized and therefore difficult to use as benchmarks for improvement of treatment [14]. Further development and application of these approaches and concepts to the pediatric population will be critical.

An understanding of the nature and characteristics of ''persister'' organisms and dormancy is fundamental to developing a reasonable strategy for shortening the duration of current tuberculosis treatment. Although the presence of a spectrum of actively replicating to nonreplicating persister

populations in active tuberculosis has been hypothesized for many years [15, 16], the role these organisms play in active human tuberculosis has not been well characterized. Much of the related research has been indirect or based on animal model studies of unproven relevance to tuberculosis in humans. The recent discovery of resuscitation-promoting factors [17], if validated, offers potential for evaluating nonreplicating persister populations in clinical studies. Appropriate in vitro and in vivo models to elucidate the activity of new drugs against the full spectrum of mycobacterial populations are critical to identifying the most-potent sterilizing combinations of drugs to shorten treatment durations.

A short, potent regimen will require antimicrobial effects against a spectrum of *M. tuberculosis* populations, and drugs targeting universal, essential microbial pathways may play a significant role. For instance, disruption of basal energy/adenosine triphosphate production needed to maintain membrane structural and functional integrity, including proton motive force, and direct disruption of components of the mycobacterial cell membrane may offer key points of intervention. New insights into the functions responsible for the viability of highly stressed cells has been gained from the recent discovery of a mechanism of action of pyrazinamide, inhibition of trans-translation [18].

A new area of research for improving and shortening the duration of tuberculosis treatment is the combination of immune-protective mechanisms in conjunction with chemotherapy to aid in the prevention of disease activation or to assist in the rapid eradication of mycobacterial populations through host immune mechanisms, especially in the setting of drug-resistant disease (M. Uhlin et al, ''Biological and adjunct immunotherapies for difficult to treat, drug resistant tuberculosis,'' *Journal* supplement, this issue). Critical to the success of the combination of defined molecular vaccines (adjuvanted protein, DNA, or virally vectored) and drug therapy is the coordination of drug and vaccine research. In addition, treatment and prevention modalities for tuberculosis may have to address host and pathogen factors alike to be successful.

THE PATH FORWARD—THE CRITICAL NEED FOR COORDINATION

The importance of enhanced communication, coordination, and, when appropriate, collaboration cannot be overstated. For example, the implementation of long-term, coordinated multisite, prospective cohorts in diverse tuberculosis-endemic countries could serve as a critical building block in future tuberculosis research efforts and scientific strategies geared toward optimizing tools and approaches in both fundamental and clinical research. Accelerated translation of fundamental scientific discoveries into clinically relevant technologies and interventions requires increased cooperation across disciplines and among various research organizations (including those in tuberculosis-endemic countries), funding agencies, implementing programs, and others that make up the global tuberculosis research community. Effective coordination efforts for tuberculosis diagnostic/biomarker, therapeutic, and vaccine research are critical at this time. The Stop TB Partnership's Working Groups and TB Research Movement are important foundations for research coordination and collaboration. Undoubtedly, a wide variety of agreements and coordinating bodies will be required to realize such a large objective, but they should all share the fundamental principle of efficient and timely sharing of information among the participating organizations. This will facilitate maximum productivity by coordinating research activities across the continuum of tuberculosis research and guarding against unnecessary bureaucratization of working relationships. The common overall goal is to advance key research objectives rapidly and cost effectively, while maintaining the highest standards of scientific rigor.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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