Update in *Mycobacterium tuberculosis* Lung Disease 2014

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The global tuberculosis (TB) pandemic continues despite intensive TB control and research efforts. Research articles related on *Mycobacterium tuberculosis* (M.tb) infection published in the *Journal* focused on five main areas: basic science, evaluation of novel diagnostics, epidemiology, transmission, and clinical trials. These range from exciting developments in new compounds to treat multidrug-resistant (MDR)-TB to major disappointments from clinical trials evaluating new treatment-shortening regimens and vaccine efficacy studies. We review these publications and discuss developments and approaches that may maximize improved TB management.

Basic Science: Immunology and Pathogenesis Studies

Comstock and colleagues demonstrated that greater tuberculin skin test (TST) reactivity, indicating "strong" immunity, also predicts the development of pulmonary TB (1). This complexity in the double-edged nature of the human immune response to M.tb is illustrated by other clinical observations. Patients with a severely impaired immune response, such as in advanced HIV infection, develop disseminated disease, but when their immune response reconstitutes will often develop immunopathology such as pulmonary cavitation. Therefore, our understanding of optimal host immune responses needs to be much more nuanced, considering both protection and pathology. Several studies published in the *Journal* addressed these complexities.

Knaul and colleagues investigated myeloid-derived suppressor cells, a subset of innate cells that can suppress T-cell responses, in the mouse model (2). Myeloid-derived suppressor cells phagocytosed M.tb and also maintained their suppressor function, while targeted depletion reduced bacterial load and pathology. A key question will be whether in humans depletion may lead to exacerbated immunopathology by removing a regulatory cell type (3). A second emerging T-cell subtype is the mucosal-associated invariant T (MAIT) cell, which recognizes vitamin B metabolites unique to pathogens presented via MHC class I–related protein 1 (MR1). Although the role of MAIT cells in animal models is established, Jiang and colleagues investigated the functional role of MAIT cells in patients with TB (4). MAIT cell numbers in patients with TB were reduced in peripheral blood and in pleural effusions, and expression of the inhibitory receptor programmed death-1 (PD-1) was increased. PD-1 blockade could improve MAIT cell cytokine production. Together, these two articles highlight the complex interplay of multiple T-cell subtypes that together contribute to the overall immune response, which in most individuals contains infection but in a small subset fails, leading to reactivation, protease-driven cavitation, and transmission (5).

Investigating patients with risk factors for TB identified various elements of an efficacious immune response. Jambo and colleagues studied lung $CD4^+$ T-cell responses in patient with asymptomatic HIV infection on antiretroviral treatment, who remain at increased risk of pulmonary TB (6). Alveolar macrophage function and mycobacteria-specific T-cell responses in adults who had been treated with antiretroviral therapy for less than 4 years were impaired compared with HIV-uninfected control subjects, thereby providing a potential mechanistic explanation for the increased TB incidence despite antiretroviral therapy. Cigarette smoking is also associated with infection, and O'Leary and colleagues studied infection of alveolar macrophages from smokers, nonsmokers, and ex-smokers with M.tb (7). In smokers, alveolar macrophage control of M.tb growth was impaired; secretion of key cytokines such as tumor necrosis factor-α, IL-1β, and IFN-γ was not significantly up-regulated; and immunosuppressive $FoxP3^+$ T cells were induced. Therefore, the smoking epidemic in resource-poor nations may induce a range of immunological deficits that can be predicted to increase M.tb infection.

From the mycobacterial perspective, M.tb must modulate the host immune response to prevent its eradication but at the same time drive tissue-destructive immunopathology to permit transmission. This long-established paradox and the role of the M.tb hypoxic gene regulator

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DosR on adaptive immunity (8) led Mehra and colleagues to investigate the role of the M.tb hypoxic gene regulator DosR on adaptive immunity in a nonhuman primate model (9). DosR mutants initially replicated but were unable to persist or cause disease, and, critically, this was associated with an increase in $CD4^+$ and $CD8^+$ T cells expressing CCR5, a marker of tissue homing. This suggests a role for DosR to inhibit the early T-cell response. Another primate study of infection with marked M.tb isolates (a sequenced panel of eight M.tb strains, each distinguished by a single nucleotide polymorphism, creating a molecular barcode for each isolate) showed that different lesions progressed at diverse rates and trajectories, demonstrating an inherent variability within the same host (10). This illustrates the need to develop model systems that can reflect this variability, incorporating M.tb and host cells within extracellular matrix under diverse physiological stresses. This presents a challenge to experimentalists who have traditionally valued reproducibility and regarded diversity of outcome as a lack of scientific robustness.

Epidemiology and Prevention of Transmission

Prevention of M.tb transmission remains the optimal control method in the absence of an efficacious vaccine. Therefore, understanding the risk factors for TB transmission and the development of latent TB infection or TB disease is critical to inform effective interventions. Seven studies analyzed various epidemiological factors in transmission. Nosocomial transmission of TB remains a major problem, and solutions must be applicable to resource-poor settings. Mphahlele and colleagues further demonstrated the efficacy of upper room ultraviolet (UV) air disinfection in reducing infectious aerosols by approximately 80%, and developed an algorithm to calculate the necessary UV exposure for any room (11). This has significant potential for the prevention of M.tb infection in health care settings. The statement that the lifetime risk of TB after exposure is 5–10% is based on historical data, and Sloot and colleagues performed a current study of 10 years of data from Amsterdam (12). Their findings suggest that the incidence of coprevalent TB, defined as disease developing within 180 days of diagnosis of the index case, is higher than previous estimates, particularly in children. In contrast, TB incidence in those with evidence of TB infection who did not receive isoniazid chemoprophylaxis was 2.4% at 5 years, suggesting the widely quoted figure of 5% may be an overestimate dating from a time of greater community transmission. However, the necessary methodological assumptions within the model mean that a different data analysis could derive a higher incidence. The study underlines the importance of contact tracing, particularly in children, to identify coincident cases of TB.

Kasaie and colleagues performed a computational modeling analysis of transmission in a moderate incidence setting and the impact of contact tracing (13). An interesting conclusion of the modeling was that, although the year-on-year 2% decline was modest, when combined with chemoprophylaxis the benefit doubled and the cumulative effects over several years would significantly contribute to TB control. In addition, the population effects were delayed for 2 years from initiation, and persisted after completion. Therefore, within the limitations of a modeling exercise, it validates contact tracing efforts combined with chemoprophylaxis and, in support of this, the predictions of the model correspond well with observations in trials.

Chan and colleagues attempted to improve the effectiveness of contact tracing by developing a simple predictive scoring system for the risk of tuberculosis in child contacts, using nationwide public health data in Taiwan (14). The eight-point score included both index case factors, such as smear positivity, sex, and residence in high-incidence areas; and contact factors such as tuberculin reaction. This method accurately predicted those individuals with the highest risk of TB, using derivation and validation cohorts, with a 100% TB risk at a score of 7 falling to a 0.2% risk from a score of 2. The incidence of TB in contacts was relatively low (0.3% for 3-yr follow-up), but such a scoring system can assist in ensuring that resources can be focused on those most in need of chemoprophylaxis. Zelner and colleagues analyzed the protective effects of isoniazid preventive therapy and bacillus Calmette-Guérin vaccination for preventing TB disease, using data from a large observational study cohort of TB contacts in Peru (15). Bacillus Calmette-Guérin was protective among children less than 10 years of age, and isoniazid was protective among contacts younger than 30 years of age. The study reconfirmed the well-established fact that the risk of TB is greatest in children under the age of 5 years, but highlighted the fact that chemoprophylaxis programs should also be expanded to include older children.

Management of contacts of patients with MDR-TB remains challenging. Fox and colleagues performed a modeling analysis of contacts of patients with MDR-TB, studying the potential outcomes of fluoroquinolone chemoprophylaxis, considering both beneficial and potentially deleterious effects (16). The model predicted that fluoroquinolone prophylaxis would be highly cost effective, reducing mortality and the incidence of MDR-TB. Somewhat counterintuitively, the model also predicted that the incidence of fluoroquinolone-resistant MDR-TB would be reduced by single-agent chemoprophylaxis, as the development of active TB and subsequent fluoroquinolone resistance would be higher without chemoprophylaxis. Evidently, the conclusions of the modeling analysis will require further evaluation in the field.

Walter and colleagues analyzed records of more than 120,000 immigrants to determine whether the high rate of TB in immigrants was due to imported active TB or reactivation of latent TB early after arrival, an important question to inform where to target screening (17). Although in the first year the vast majority of TB was imported, based on preimmigration examination data, explaining the high incidence in the year after arrival, in Years 2–9 TB cases were predominantly the result of reactivation of latent TB. Of note, the rate of reactivation (32 per 100,000) did not decline over the 9-year period, suggesting that screening and treatment should be offered even beyond 5 years after immigration in low-incidence countries. The authors also concluded that ultimately the best way to reduce imported TB is to assist high-incidence countries to successfully control TB. This will require coordination between both communicable and noncommunicable disease programs as the two epidemics progressively converge (18).

Evaluation of *Mycobacterium tuberculosis* Diagnostics

The rollout of the Xpert MTB/RIF assay system (Cepheid Diagnostics, Sunnyvale, CA) has been a key change in TB diagnostics over the past 5 years. Davis and colleagues

performed analysis of data from a cross-sectional study of 156 patients and studied hypothetical changes in management (19). They concluded that Xpert-guided management could reduce unnecessary empiric treatment and contact investigation, thereby permitting more targeted allocation of resources. Hanrahan and colleagues studied the use of Xpert cycle threshold (C_T) values as a measure of mycobacterial burden (20). The C_T correlated with time to culture positivity, meaning that the C_T value may be a surrogate of the level of infectiousness and guide contact tracing exercises. Interestingly, increasing levels of HIV-related immunosuppression were associated with lower bacillary burden, potentially because an intact immune response is required to drive cavitation and subsequent exponential mycobacterial growth.

Innovative new approaches for microscopic diagnosis are emerging. Ismail and colleagues evaluated performance of an automated digital microscopy platform with digital image processing, using more than 1,000 samples (21). They found performance to be equivalent to that of an experienced TB microscopist, and that it provided acceptable sensitivity and a high specificity for active TB. Furthermore, using "low positive" scores, defined as one to nine putative acid-fast bacilli detected, as initial screening significantly reduced the number of Xpert analyses performed. In TB meningitis, diagnosis is often a challenge. Feng and colleagues studied the accuracy of a protocol staining intracellular M.tb within leukocytes or an early secretory antigen target (ESAT)-6 immunohistochemical stain of cerebrospinal fluid cells on Cytospin slides (22). A simple modification of the Ziehl–Neelsen stain on cerebrospinal fluid cells on Cytospin slides increased sensitivity from 3.3% for conventional staining to 82.9%, demonstrating great potential to increase the early diagnosis of TB meningitis. Further optimization will be required because of the relatively high number of apparent false positives (15%).

There have been several studies of IFN-γ release assays (IGRAs) in detecting latent M.tb infection and their usefulness in predicting development of active TB disease. Zellweger and colleagues studied the role of IGRAs to predict the development of TB in recent contacts in Europe (23) and concluded that, although the negative predictive value of IGRAs is high, IGRAs do not reliably predict the development of active TB. Dorman and colleagues studied more than 2,400 health care workers longitudinally, comparing QuantiFERON-TB Gold In-Tube and T-SPOT.*TB* with the TST (24). Many more participants converted from noninfected to M.tb infected as assessed by IGRAs than by the TST, but the majority of conversions appeared to be false positives as they were frequently negative when retested 6 months later. These conversions in part reflect the arbitrary nature of the manufacturers' definition of the threshold for a positive test. Therefore, in low-incidence countries, testing should be targeted only at those at highest risk (25).

In South African adolescents, Andrews and colleagues similarly studied the longitudinal dynamics of IGRAs (26). Concordance between IGRA and TST conversion was high, and the authors concluded that the predictive value of conversion demonstrating M.tb infection is greater in high-incidence settings than in low-incidence settings. TST conversion was defined as 5 mm for a positive test. IGRA reversion occurred in more than 20%, but these individuals still had the same TB incidence as those whose IGRA remained positive, suggesting that in a proportion of subjects infection occurred and the second IGRA was a false negative. In children, in whom TB diagnosis is particularly challenging, Mandalakas and colleagues found in a high-burden setting that both TST and IGRAs correlated with TB contact, but that the IGRAs has a closer correlation than TST (27). QuantiFERON-TB indeterminate tests were more frequent in children infected with HIV, whereas T-SPOT.*TB* had a lower indeterminate rate unaffected by HIV status, and so T-SPOT.*TB* may be the assay of choice where resources permit.

The optimal screening approach for patients who are immunocompromised has not yet been defined. The TBNET consortium compared TST and IGRA performance in five groups of immunocompromised patients (28). The study was limited by generally low TB incidence, with only 11 patients in the cohort developing TB, and these were predominantly HIV infected. Progression to TB was poorly predicted by either TST or IGRAs, highlighting the need for better biomarkers to target preventive chemotherapy to the right populations (29).

Whereas the role of IGRAs has been systematically investigated, the role of unbiased microarray-based tests is only emerging. In three separate African child cohorts, Anderson and colleagues demonstrated that host transcriptome responses could be used to differentiate tuberculosis from other diseases, based on a signature of 51 gene transcripts (30). Although promising, the challenge will be to develop technology that can be applied in the field with sufficient sensitivity and specificity and fulfilling key criteria such as low cost, minimal user training, and disposable reagents.

Clinical Trials

It is an enduring irony that currently recommended "short-course" TB treatment regimens last for 6 months. Genuine short-course therapy would be transformative, improving patient adherence and reducing cost. Mortality in hospitalized patients remains unacceptably high (31), meaning that clinical trials should not only focus on treatment shortening and drug-resistant disease, but also on patients presenting with severe disease who succumb to immunopathology early in treatment. Studies published can broadly be divided into those studying (*1*) interventions currently available but modified for more effective use, (*2*) novel TB treatment–shortening regimens, (*3*) treatment of multidrug-resistant disease, (*4*) biomarkers of treatment response, and (*5*) targeting of the host (host-directed therapies).

More Effective Use of Current Interventions

Rifampin is critical to the standard TB treatment regimen. The standard dose of 10 mg/kg was chosen more than 40 years ago, in part because of cost considerations. Boeree and colleagues reconsidered rifampin in a dose-ranging trial to investigate the safety and tolerability of increasing doses (32). Progressive groups received rifampicin in increasing doses up to 35 mg/kg for 14 days. Even at the highest dose, rifampicin was safe and well tolerated and generated even higher plasma concentrations than predicted, due to nonlinear pharmacokinetics. The bacterial load fell most in the highest dose groups. The later time points in treatment were not reported, and so the longer term effects cannot be determined (33). Follow-on studies are ongoing.

A similar strategy was investigated by Dorman and colleagues, who studied high-dose daily rifapentine (34), which may have potential benefits of a longer half-life and lower minimal inhibitory concentration than rifampin. In a study of 334 participants, those receiving high-dose rifapentine (20 mg/kg) had significantly higher sputum sterilization compared with the rifampin control group, suggesting that high-dose rifapentine may achieve more rapid sterilization. It remains to be determined which rifamycin will be optimal, and how well higher doses are tolerated within a programmatic setting.

In South African goldmines, a study of mass screening and treatment with isoniazid chemotherapy was performed, with more than 23,000 miners prescribed isoniazid for 6 months (35). Unfortunately, the intervention did not reduce the incidence of tuberculosis, even though during the period of isoniazid treatment it was reduced. In contrast, Rangaka and colleagues studied isoniazid chemoprophylaxis in people infected with HIV receiving antiretroviral treatment and demonstrated a reduction in the incident cases of TB (36). Of note, the benefit of isoniazid preventive therapy did not associate with either a positive IGRA or tuberculin skin test, indicating that in high TB incidence settings, all HIV-positive patients receiving antiretroviral therapy should also receive isoniazid chemotherapy irrespective of tests of prior TB exposure.

In those patients with HIV who develop active TB, the optimal timing of initiation of antiretrovirals is not fully defined. Although it is well established that patients with very low CD4 counts (<50 cells/μl) benefit, a study of patients with CD4 counts exceeding 220 cells/μl demonstrated that the initiation of antiretroviral therapy can be delayed until after the completion of TB treatment without any evidence of increase in adverse outcomes (37). This will simplify treatment and reduce the pill burden.

Novel TB Treatment Regimens

Novel drug combinations were investigated by Diacon and colleagues using combinations of pyrazinamide and clofazimine with pretomanid and bedaquiline (38). Daily sputum culture performed over the first 14 days of treatment identified bedaquiline, pretomanid, and pyrazinamide as a novel regimen, with equivalent efficacy to the current standard four-drug regimen. In contrast, clofazimine did not add to the activity of the other drugs. Longer studies will now be required to determine how the early bactericidal activity correlates to the late sterilizing action that will be required for treatment-shortening regimens (39).

Three major trials examined whether adding a fluoroquinolone in a modified TB regimen could shorten treatment to 4 months: REMoxTB (Rapid Evaluation of Moxifloxacin in Tuberculosis; 40), the OFLOTUB (Ofloxacin-Tuberculosis) project (41), and RIFAQUIN (rifapentine and a quinolone; 42). The noninferiority trial design generated the conclusion that each 4-month regimen was not noninferior, despite greater sputum culture conversion at 2 months. Clearly this outcome after major investment of resources and time is highly disappointing. The results mean that the model systems that underpinned this approach have failed to adequately predict outcomes in humans. Drug development traditionally relies on the sequence of the three M's (minimal inhibitory concentration, mouse, and man), but these studies suggest that alternative models are required, reflecting the complexity of the host–pathogen interaction *in vivo*. The biology of persister M.tb bacilli that cause relapse, most likely slowly replicating, physiologically resistant or "viable but nonculturable" M.tb bacilli, needs to be understood, to inform interventions that kill these bacilli and permit successful treatment-shortening regimens.

Treatment of Multidrug-Resistant TB

Data published from MDR-TB were more promising. Diacon and colleagues reported a phase 2b study in which bedaquiline (TMC207) was given for 24 weeks to 160 patients with MDR-TB, resulting in reduced time to culture conversion and increasing cure rate at 120 weeks from 32 to 58% (43). The World Health Organization has published guidelines on the use of delamanid and bedaquiline for the treatment of MDR-TB (44). However, the bedaquiline trial still left more than one-third of patients not cured even with this improved regimen. Heyckendorf and colleagues reviewed the knowledge needed to predict optimal duration of therapy for MDR and extensively drug-resistant (XDR)-TB as opposed to the current standard of 20 months, and the biomarkers that may inform clinical decision making (45).

Biomarkers of Treatment Response

Biosignatures of treatment response can be from the pathogen or host, and all markers are still in the early phase of evaluation. However, the application of emerging unbiased technologies, such as genomics, proteomics, and metabolomics, should accelerate progress if applied systematically to the appropriate clinical specimens. These biosignatures can be integrated with novel clinical trial designs, such as that proposed by Nunn and colleagues (46), to accelerate the rate of progress.

Host-Directed Therapies

Because of the continuing high mortality associated with the TB epidemic, and the lengthy duration of treatment, recent attention has refocused on "host-directed therapies." A range of "host factors" can alter the human body's immune system and are responsible for poor treatment response or increased mortality. Murray suggested a study of high-altitude sanitoria for XDR-TB to reevaluate this classical treatment (47). Traditionally, TB treatment has focused on mycobacterial killing with TB drugs, but the concept of host-directed therapy to improve outcomes in TB is emerging (48). Host-directed therapies may fall into a wide range of categories (49) and include interventions with immune-modulatory effects, or drugs that can inhibit macrophage intracellular signaling pathways, inhibit matrix metalloproteases, or enhance autophagy. More recent studies include a trial of either steroids or immunotherapy using environmental mycobacteria for the treatment of TB pericarditis, as reported by Mayosi and colleagues (50). In a study of 1,400 patients, adults with TB pericarditis received either prednisolone or *Mycobacterium indicus pranii* immunotherapy, as five injections over 3 months. There was no significant difference in the primary composite outcome (death, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis) between prednisolone and placebo, or between *M. indicus pranii* and placebo. However, prednisolone did significantly reduce the incidence of constrictive pericarditis and hospitalization, albeit with an increased risk of HIV-associated cancer. Skrahin and colleagues reported an open label phase 1 safety trial

of autologous bone marrow–derived mesenchymal stromal cell infusion for patients with MDR- and XDR-TB (51). The intervention was safe and a phase 2 trial is underway. Host-directed therapy is a rapidly evolving field, and commonly used drugs for arthritis, diabetes, epilepsy, and cancer could be "repurposed" for reducing destructive inflammation that skews the host–pathogen interaction in favor of M.tb (52, 53).

Conclusions

Several important studies have increased our knowledge of TB epidemiology, diagnosis, and management. New innovations for the development of more effective point-of-care diagnostics, shortening the duration of therapy, and improving treatment outcomes remain urgent research priorities. However, the unsuccessful vaccine and treatment-shortening trials may suggest that approaches diverse from the mainstream need to be pursued. These alternative approaches require funding of individual innovators pursuing highly innovative and paradigm-challenging research programs. In parallel, the rapidly emerging unbiased "-omic" technologies such as genomic and proteomic profiling present great opportunity. A range of host-directed therapies (HDTs) for improving treatment outcomes requires evaluation in randomized clinical trials as adjuncts to current TB treatment regimens. Applying such technologies and performing a large number of clinical trials will require the development and funding of major consortia drawing on diverse multidisciplinary expertise in parallel with funding fundamental research to fill key knowledge gaps. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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