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REVIEW

Incipient and Subclinical Tuberculosis: a Clinical Review of Early Stages and Progression of Infection

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SUMMARY Tuberculosis (TB) is the leading infectious cause of mortality worldwide, due in part to a limited understanding of its clinical pathogenic spectrum of infection and disease. Historically, scientific research, diagnostic testing, and drug treatment have focused on addressing one of two disease states: latent TB infection or active TB disease. Recent research has clearly demonstrated that human TB infection, from latent infection to active disease, exists within a continuous spectrum of metabolic bacterial activity and antagonistic immunological responses. This revised understanding leads us to propose two additional clinical states: incipient and subclinical TB. The recognition of incipient and subclinical TB, which helps divide latent and active TB along the clinical disease spectrum, provides opportunities for the devel-

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opment of diagnostic and therapeutic interventions to prevent progression to active TB disease and transmission of TB bacilli. In this report, we review the current understanding of the pathogenesis, immunology, clinical epidemiology, diagnosis, treatment, and prevention of both incipient and subclinical TB, two emerging clinical states of an ancient bacterium.

KEYWORDS *Mycobacterium tuberculosis*, tuberculosis

INTRODUCTION

Mycobacterium tuberculosis is an ancient bacterium that was first presented as the cause of tuberculosis (TB) in 1882. Despite the global introduction of a vaccine and the discovery of an effective four-drug treatment regimen, *M. tuberculosis* still likely infects approximately one-quarter of the world's population and is the leading infectious cause of mortality worldwide (1, 2). The current TB pandemic is fueled not only by poverty and HIV/AIDS but also by an insufficient understanding of the spectrum of TB pathogenesis, which may be essential in developing new diagnostic tests and creating more-adaptable treatment regimens.

The "End TB Strategy" of the World Health Organization (WHO) seeks to reduce TB incidence to fewer than 10 cases per 10^5 people per year by 2035 (3). The primary approach for achieving this goal is to improve efforts to find and treat people with active TB disease, conduct universal screening of individuals at high risk, and provide preventive therapy for those at risk of progressing to active TB disease (3). While there are concentrated efforts to develop a new vaccine, this alone may not be enough to end the TB epidemic (4). A strong public health response will require new diagnostic tools and therapeutic options along with a better understanding of the microbiological and clinical spectrum of TB infection and disease.

The Spectrum of Tuberculosis Infection to Disease

A longstanding tenet of TB pathogenesis has been that *M. tuberculosis* exists in either a metabolically inactive latent state or a metabolically active disease state. In this framework, about 5% of people infected with TB progress rapidly to active disease, while the vast majority of people develop a latent infection and remain at risk for progression to active disease ("reactivation") (5). Studies of pathogenesis in animal models and in humans suggest a more complex course, where, following initial contact between *M. tuberculosis* and a human host, the pathogen may progress to primary active TB disease or be completely eliminated through an innate and/or acquired immune response (4). For individuals with latent TB infection, the host maintains a dynamic relationship with *M. tuberculosis* through the regulation of available nutrients as well as the innate and acquired immune systems (6, 7).

This new paradigm for a dynamic continuum of *M. tuberculosis* infection suggests that additional categories of infection can be defined between latent infection and active TB disease (8). With a better understanding of the TB spectrum, we propose and describe two additional clinical states, called incipient and subclinical TB. Although the spectra of TB infection and disease remain continuous, categorizing *M. tuberculosis* into these discrete states may allow for better dialogue on TB pathogenesis and help foster the development of new diagnostic and therapeutic interventions to prevent progression to active TB disease and ongoing transmission of *M. tuberculosis* bacilli.

DEFINITIONS OF INCIPIENT AND SUBCLINICAL TUBERCULOSIS

We propose a new framework in which the pathophysiological spectrum is divided into five discrete categories for both conceptual and practical purposes. This five-state framework incorporates a recent understanding of clinical TB pathogenesis as well as advances in the diagnostic approaches to detecting viable TB and predicting the progression of disease activity. These definitions and categories are meant to build upon each other, as detailed in Table 1.

Eliminated TB infection is an individual with prior exposure to *M. tuberculosis* who either has cleared the infection by innate and/or acquired immune responses or has

TABLE 1 Defining criteria for the five categorical states of tuberculosis

Categorical state of TB	Presence of criterion				
	M. tuberculosis exposure	Person has viable *M. tuberculosis* pathogen	*M. tuberculosis* has metabolic activity to indicate ongoing or impending progression of infection	Radiographic abnormalities or microbiological evidence of active, viable *M. tuberculosis*	Person has symptoms suggestive of active *M. tuberculosis* disease
Eliminated TB infection	×				
Latent TB infection	×	×			
Incipient TB infection	×	×	×		
Subclinical TB disease	×	×	×	×	
Active TB disease	×	×	×	×	×

been cured of the infection with anti-TB medications. This individual no longer has viable *M. tuberculosis* bacteria but may still have immunological evidence of prior infection.

Latent TB infection (LTBI) is infection with viable *M. tuberculosis* for which progression to TB disease is not expected to occur in the near future in the absence of any significant immunological compromise. This represents a more conceptual analogue to the current WHO definition, which considers LTBI "as having evidence of TB infection and no clinical, radiological or microbiological evidence of active TB disease" (9–11). Currently, there is no direct way of confirming LTBI or its microbiological load, as existing tests infer LTBI based on a T cell response to TB or TB-like antigens (12).

Incipient TB infection is an infection with viable *M. tuberculosis* bacteria that is likely to progress to active disease in the absence of further intervention but has not yet induced clinical symptoms, radiographic abnormalities, or microbiologic evidence consistent with active TB disease.

Subclinical TB disease is disease due to viable *M. tuberculosis* bacteria that does not cause clinical TB-related symptoms but causes other abnormalities that can be detected using existing radiologic or microbiologic assays.

Active TB disease is disease due to viable *M. tuberculosis* that causes clinical symptoms with radiographic abnormalities or microbiologic evidence consistent with active TB disease. This would remain consistent with the current WHO definition, which considers active TB disease as "symptomatic patients with radiological or microbiological evidence of *M. tuberculosis*" (13).

Following the establishment of latent infection, the pathways by which disease may naturally progress include (i) latency (the most common course, which encompasses persistent or eliminated disease burden), (ii) rapid or (iii) slow progression through incipient and subclinical disease to active TB, or (iv) a period of cycling through incipient and subclinical states that may precede the development of symptomatic disease or eventual disease resolution (Fig. 1). A larger disease burden may correlate with increasing host symptoms, a likelihood of transmission, a decreasing probability of spontaneous self-cure, an impaired or poor immunological response, and/or a larger abundance of TB bacilli and other pathogen biomarkers. While we have not depicted all possibilities for regression of disease burden, spontaneous recovery may occur in any of these clinical trajectories, including pathways that have progressed through active TB.

We acknowledge that there are limitations by applying discrete categorical definitions to a continuous process. First, this introduces challenges with categorical interpretation, particularly as people transition from one state to another. As such, the time spent within each disease state as well as routes of progression may be highly variable. Second, there may also be various levels of evidence for patients to fit these categorical definitions. For example, a culture with *M. tuberculosis* may be a higher level of diagnostic evidence for subclinical TB than chest radiography suggestive of upper lobe disease. Despite these limitations, we hope that applying new categories to the continuous spectrum of TB will help advance biomedical research and interventions to end the TB epidemic.

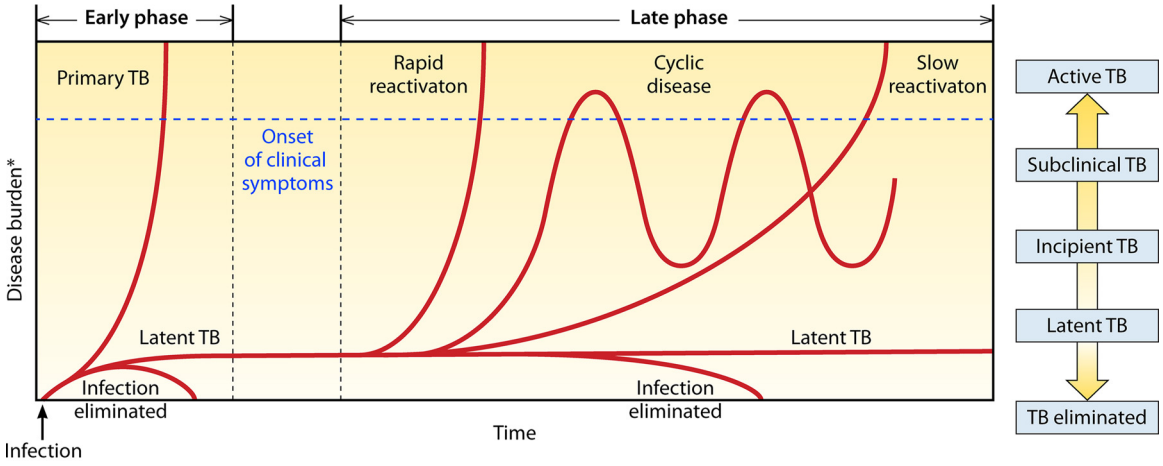

FIG 1 Pathways of tuberculosis disease progression. After initial exposure, *M. tuberculosis* may be eliminated by the host immune response, persist as a latent infection, or progress to primary active disease. Following the establishment of latent infection, disease may persist in a latent form, naturally progress in a slow or rapid fashion to active tuberculosis, or cycle through incipient and subclinical states before developing into symptomatic disease or eventual disease resolution. Although not all possibilities for regression of disease burden are depicted, spontaneous recovery may occur in any of these clinical trajectories.

BACTERIAL PATHOGENESIS AND IMMUNE RESPONSE

Pathogenesis of Incipient and Subclinical Tuberculosis

M. tuberculosis exerts a wide spectrum of infectious pathophysiology. Evidence is mounting that genetic and phenotypic variation of the bacteria, along with the interaction between these bacteria and their individual hosts, can influence the progression of TB. To our knowledge, very few studies have focused specifically on the state of the pathogen during incipient TB or subclinical TB or progression through these states. Therefore, much of this section relies on making inferences from studies dichotomously conceptualizing latent TB infection and active TB disease.

At the population level, genetic heterogeneity between *M. tuberculosis* strains influences interactions with the host immune system and the consequent pathogenesis of TB infection. *M. tuberculosis* strains show substantial variation in their virulence and immunogenicity, which ultimately impact their propensity to induce or accelerate active disease (14–17). Based on whole-genome sequence analysis, human-adapted *M. tuberculosis* complex strains have been divided into seven phylogenetic lineages (15–20). Lineages 2 to 4, which harbor a deletion in the genomic region known as TbD1, are collectively referred to as "modern" because these strains diversified genetically more recently than the "ancient" lineages (lineages 1, 5, and 6) and "intermediate" lineage 7 (17, 18, 21). Numerous differences in host disease presentation have been ascribed to the different lineages, some of which are listed in Table 2. In addition, mechanistic studies have shown that different *M. tuberculosis* strains induce differential innate and adaptive immune responses via multiple effectors (16–18). Modern *M. tuberculosis* strains have been shown to elicit lower-level and delayed proinflammatory cytokine production from human peripheral blood mononuclear cells and replicate more rapidly in aerosol-infected mice and are more pathogenic to mice than more-ancient TB strains (17, 18, 20, 22–24). A well-characterized example is the HN878 strain of the lineage 2 Beijing family, which is highly virulent in mouse and rabbit models and has been implicated in multiple human TB outbreaks (25–27). HN878 produces a particular phenolic glycolipid in its cell wall (28, 29) and induces altered innate and adaptive immune responses (see Table 3 for examples). Together, these processes result in increased lung pathology and reduced survival in immunocompetent mice. In contrast, infections with ancient lineage 6 strains result in reduced bacterial burdens in marmosets and differential T cell responses in humans compared to modern-lineage

TABLE 2 Summary of *M. tuberculosis* complex phylogenetic lineages, including some of the reported differences in disease presentation

Lineage	Geographic distribution	Main lineage-specific genetic deletion(s)	Difference(s) in disease presentation	Reference(s)
1 (Indo-Oceanic)	East Africa and Central, South, and Southeast Asia	RD239	Elicits higher IFN-γ responses than lineage 2 and 3 strains; not associated with extrapulmonary disease	237, 238
2 (East Asian [includes Beijing family])	Central and East Asia, Eastern Europe, and South Africa	TbD1, RD105	Associated with extrapulmonary disease; induces more-rapid wt loss than lineage 6; associated with relapse, treatment failure, and fever early during treatment	30, 238–242
3 (East African-Indian)	East Africa and Central, South, and Southeast Asia	TbD1, RD750	Associated with extrapulmonary disease; higher-level anti-inflammatory phenotype than with lineage 4	243
4 (Euro-American)	Asia, Europe, Africa, and the Americas	TbD1, pks15/1	Associated with more α1-acid glycoprotein and C-reactive protein, higher neutrophil counts, and lower body mass index than with lineage 1; associated with more pulmonary than meningeal disease; lower mortality rates from meningeal TB; associated with lung consolidation in chest X ray	32, 244, 245
5 (West Africa [*Mycobacterium africanum*])	West Africa	RD9, RD711	Lower rate of progression to disease than for *M. tuberculosis*	32
6 (West Africa [*M. africanum*])	West Africa	RD9, RD7, RD8, RD10, RD702	Lower rate of progression to disease than for *M. tuberculosis*; more extrapulmonary spread	30–32
7 (Ethiopia)	Ethiopia	RD3, RD11, 10 bp in *mmpL9*, 27 bp in *lppH*, 1.3 kb in *lppO-sseB*, 3.3 kb in *Rv3467-rmlB2-mhpE*	Delay in patients seeking treatment	246

strains and have been associated with reduced virulence and reduced progression to disease (30–32). As a result, it has been proposed that as human populations expanded rapidly, *M. tuberculosis* may have evolved traits of more-rapid disease progression and increased transmission (16–18). Given the influence of different *M. tuberculosis* strains on the host immune response and disease progression, the state of the pathogen is likely to play an important role in shaping the disease states for incipient and subclinical TB.

Within the context of an individual patient, different infection sites within the lung create diverse microenvironments that induce bacterial phenotypic heterogeneity, which can in turn influence the immune response and progression of infection, from LTBI through the spectrum of incipient and subclinical TB and, ultimately, to active TB disease. Variations in the cellular compositions and activation levels of immune cells,

TABLE 3 Examples of altered immune responses by *M. tuberculosis* strain HN878 relative to the CDC1551 or H37Rv strain

Immune response	HN878-induced difference(s) vs H37Rv or CDC1551	References
Innate	Altered interactions with pattern recognition receptors (e.g., Toll-like receptors) Reduced activation of monocytes, macrophages, and dendritic cells	29, 247, 248
Adaptive	Raised levels of $CD4^+/CD25^+/FoxP3^+/CD223^+/IL\text{-}10^+$ regulatory T cells	27, 29, 249
Cytokine	Induction of type I IFNs Induction of T_H2 cytokines IL-4 and IL-13 Rapid repression of an initial spike in T_H1 cytokines TNF-α, IL-6, IL-12, and IFN-γ	27, 29, 249

epithelial cells, and the extracellular matrix that comprise granulomas expose the residing *M. tuberculosis* bacteria to differences in nutrient availability, reactive intermediates, and cytokine profiles as well as to differential drug penetration (5, 33–35). *M. tuberculosis* also reaches extrapulmonary sites even during asymptomatic infections, each with its own diverse milieu (36–39).

The types of lesions detectable in asymptomatic individuals correlate with the onset of subclinical TB disease and with progression toward active disease. One study surveying LTBI in individuals with asymptomatic HIV-1 infection with no clinical evidence of TB showed that over 70% of individuals harbored a broad range of abnormal lung lesions detected by [^{18}F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) combined with computed tomography (PET/CT) imaging. Importantly, individuals with subclinical TB that harbored fibrotic scars or infiltrates or who were FDG active by PET/CT were more likely to develop active, symptomatic TB disease during a 6-month follow-up period (40, 41).

The mechanisms by which *M. tuberculosis* adapts to the diverse microenvironments created by these infection sites could contribute to differential disease progression (33, 34). Experiments *in vitro* and in mouse models have characterized molecular responses to some of the specific stresses encountered by *M. tuberculosis* during infection (e.g., oxygen and nutrient limitation) that promote a physiological shift toward reduced replication (33, 42, 43). Exposure of *M. tuberculosis* to either low oxygen or starvation *in vitro* triggers large-scale changes in gene and protein expression (44–47), which remodel key cellular processes, including energy metabolism (45, 47–49). Several mechanisms underlying the *M. tuberculosis* response to these stresses enact transcriptional, posttranslational, or allosteric control (50–60). How these responses integrate to influence patient-level outcomes for incipient or subclinical TB disease is currently unclear.

Recent *in vitro* studies have shown that host stresses encountered during infection will also influence the cell-to-cell variability in the growth and metabolism of *M. tuberculosis*, creating a spectrum of phenotypically heterogeneous subpopulations even under uniform conditions (61, 62). One subpopulation with potentially high clinical relevance is a subset of cells that are metabolically viable but do not replicate, even after being returned to growth-permissive culture conditions. Phenotypically similar subpopulations of "differentially culturable tubercle bacilli" (DCTB), which could not form CFU when cultured on standard solid media but could be grown after exposure to liquid media supplemented with fresh *M. tuberculosis* culture filtrates, have been found in sputum samples of TB patients and sputum culture-negative, smear-negative individuals (63). The DCTB subpopulation appears to be phenotypically heterogeneous, as different subsets of DCTB replicate differentially when exposed to medium supplementations of resuscitation-promoting factors. The correlation between relative abundance levels of DCTB and HIV status in TB patients suggests an interaction between these bacilli and the immune response (64). However, additional studies are required to clarify the molecular mechanisms that generate DCTB and the consequences of this population in patients with a clinical phenotype of incipient or subclinical TB.

Bacterial heterogeneity also influences immune control. Primate and human experiments have shown diverse trajectories for individual granulomas (5, 35). In one study, the numbers of viable *M. tuberculosis* bacilli within individual granulomas varied by several orders of magnitude in both asymptomatic and symptomatic individuals, the histopathology of lesions correlated with the bacterial load, and individuals in both groups harbored granulomas that were completely clear of *M. tuberculosis* bacilli (65). Individuals who progressed to active disease differed from those with sustained asymptomatic infection in three key ways: (i) they had a subpopulation of lesions that exhibit a minimal bactericidal effect after the onset of adaptive immunity, (ii) they exhibited evidence of bacterial dissemination to form new granulomas at later time points, and (iii) they had increased inflammation in their lesions (5, 65). Thus, various host-directed therapies that could contain metabolically active and dividing *M. tuber-*

culosis bacilli within the existing granulomas and prevent escape may help stop disease progression (66).

Immune Correlates of Incipient and Subclinical Tuberculosis

A common feature of immune correlate studies has been the upregulation of interferon (IFN) signaling, decreased B and T cell signaling, and increased myeloid cell signaling, including phagocytosis. When analyzed kinetically, IFN signaling preceded all other transcriptional modules, suggesting the promise of an IFN-driven signature for the progression of incipient TB (67). Cell-specific validation experiments suggested that immune signatures may be attributed to neutrophils. To address the specificity of host transcriptional signatures for TB, these preliminary signatures have also been evaluated in sarcoidosis, pneumonia, systemic lupus erythematosus, and lung cancer. While some overlap was observed, four studies were able to develop a refined signature driven by IFN signaling that could possibly delineate TB from other inflammatory conditions that contained IFN-stimulated genes (68–71). However, these signatures remain to be externally validated by others.

Humoral immunity may provide protective immunity to *M. tuberculosis* (72–74). Circulating antibodies to immunodominant antigens of *M. tuberculosis* may serve as biomarkers of subclinical TB (75, 76). For example, in an antibody screening study, distinctive Fc effector functions and glycosylation patterns were strongly associated with active TB, compared to LTBI (74). Notably, pooled IgG antibodies from latently infected individuals, but not those with active TB, were able to contain *M. tuberculosis* in an *in vitro* macrophage culture assay. Low levels of *M. tuberculosis*-targeting IgG may foreshadow a transition through incipient and subclinical TB. Since humoral immunity is involved in the response to *M. tuberculosis* infection, quantification of families of antibodies may provide an approach to identifying subclinical TB.

Interferon gamma release assays (IGRAs), which measure *M. tuberculosis*-specific $CD4^+$ T cell immunity, have value in discerning exposure to *M. tuberculosis* but fail to identify infection or to distinguish the disease stage. However, one study reported that an IGRA conversion value of >4 IU/ml in infants was associated with a 42-fold-elevated risk of progression to TB (77). The presence of interleukin-2 (IL-2) cytokine-secreting $CD4^+$ T cells has been shown to correlate with bacterial burden and clinical disease (78, 79). People with active TB disease also have a more activated and proapoptotic phenotype of antigen-specific $CD4^+$ T cells than people with LTBI (80, 81). Th17-type $CD4^+$ T cell responses may be associated with a lower risk of progression to TB (67). Individuals with a lower risk for TB progression, as measured by an RNA host signature, had higher levels of Th17-associated transcripts, including IL-17F (67). Measuring T cell immune responses using both $CD4^+$ and $CD8^+$ cells may be capable of differentiating LTBI from subclinical or active TB, if the frequency of antigen-specific $CD8^+$ T cells can serve as a reflection of the antigen load and disease burden. Two independent studies found $CD8^+$ T cells at a higher frequency in smear-positive than in smear-negative active TB (79, 82). Furthermore, frequencies of TB-specific $CD8^+$ T cells are increased in disease progression in nonhuman primates (83) and decreased with anti-TB treatment (79, 84). IFN-γ production from $CD8^+$ T cells can provide a signature of TB in young children (85). Most studies have not delineated T cell immune responses by different TB disease states, incipient and subclinical TB.

As *M. tuberculosis* changes its gene expression in response to host immune pressure, the antigen repertoire may also change (86). A number of antigens, DosR-regulated proteins, and enduring-hypoxic-response proteins have been described as being selective for LTBI, but data from confirmatory studies have been inconsistent (87). At present, relatively little is known regarding antigens that are selectively expressed during early TB as well as progression of TB infection (88–90). Ag85B is highly expressed during early *M. tuberculosis* infection and appears to be repressed during chronic *M. tuberculosis* infection (91–93). Lung-specific antigens could also be of value, but only a few reports have defined these antigens (94, 95). Nevertheless, an additional under-

standing of antigen recognition by the human cellular immune system may be helpful for generating diagnostic tools for incipient and subclinical TB.

Nonclassically restricted T cells use invariant restriction molecules and can present peptidic and nonpeptidic antigens, including glycolipids (96) and TB-derived products (97, 98). HLA-E-restricted $CD8^+$ T cells can recognize TB-derived peptides (98–100) and proliferate during active TB, compared to healthy controls (101). Frequencies of CD1b-c-restricted T cells, which recognize lipid antigens, are similarly increased in during LTBI, compared to TB-uninfected individuals (102, 103). In contrast, frequencies of MR1-restricted T cells, which recognize riboflavin biosynthetic products, are reduced during active TB, compared to LTBI (104–106). Frequencies of natural killer T (NKT) cells, restricted by CD1d and recognizing glycolipid antigens, are similarly reduced from the circulation of individuals with TB compared to healthy controls (107). In a prospective household contact study, NKT cells were significantly less prevalent in people who progressed to active TB than in people who did not progress to active TB (108). Cumulatively, circulating frequencies of nonclassically restricted T cells change in the context of the progression from LTBI to active TB disease and could be useful as biomarkers to discern bacterial burdens for incipient and subclinical TB.

Proteomic, metabolomic, and epigenetic data may also be used to define signatures of TB progression (109–114). A longitudinal analysis of soluble biomarkers of an adolescent cohort monitored for progression to TB suggested that the complement cascade was the earliest upregulated group of proteins and correlated with an upregulated IFN-driven host RNA signature (67). While few of these signatures have been prospectively validated, multiple approaches suggest that measurement of the host response to mycobacterial infection can serve to identify those with subclinical and incipient TB. Future technological advances will likely define specific immune subsets associated with both incipient and subclinical TB, but validation of these approaches will need to rely upon prospective clinical trials.

EPIDEMIOLOGY OF INCIPIENT AND SUBCLINICAL TUBERCULOSIS

Our limited understanding of incipient and subclinical TB at the population level reflects the dearth of available diagnostic tools. Tests that have been utilized to understand the epidemiology of incipient and subclinical TB include national prevalence surveys, active case-finding studies, and systematic screening of high-risk groups, including preventive-therapy studies and TB vaccine trials (115–138). Studies recording both patient symptom histories and diagnostic test results, as well as postmortem studies, have also helped to characterize the burden of subclinical TB in specific populations (139, 140).

Given that incipient TB is not detectable on the basis of symptoms, radiography, or microbiological testing, our understanding of the epidemiology of incipient TB is very limited at present. We are, however, able to make some inferences about this disease state. Assuming that all cases of active TB progress through a period of meeting the definition of incipient TB, the incidence must be at least that of active TB (i.e., an estimated 10.4 million cases in 2016 [141]). To the extent that some individuals with incipient TB do not progress to detectable active disease, the incidence of incipient TB would be expected to be even higher than that of active TB disease.

The reported prevalence of subclinical TB varies widely across epidemiological settings, populations, and screening tools used. The prevalence of subclinical TB is generally high in studies performing active case finding among high-risk groups, during which all participants are screened with high-sensitivity tests (119, 123), compared to broader prevalence surveys, during which sputum culture might be performed only for persons with chest radiography suggestive of TB (142). A review of 12 national prevalence surveys in Asia found that the percentages of all bacteriologically confirmed TB cases who did not report TB symptoms (often requiring cough with a duration of 2 to 3 weeks) ranged from 40% in Pakistan to 79% in Myanmar. In three countries with repeat survey data on symptoms (Cambodia, Republic of Korea, and China), the percentages of confirmed prevalent cases who did not report symptoms rose between

18 and 21% over a period of 5 to 10 years (115, 143–145). This suggested that traditional TB control measures may reduce the prevalence of symptomatic TB but not that of subclinical TB, which may increase the proportion of subclinical TB cases among all culture-positive TB cases.

Groups at high risk for subclinical TB are likely to be similar to those for active TB and include residence in high-incidence settings; persons with a history of TB (119, 123, 139); HIV-positive individuals, particularly those not treated with antiretroviral therapy (118–123, 125–134, 146–154); pregnant and postpartum women (135, 136, 148, 155); people with noncommunicable disease comorbidity, including renal disease and diabetes (139); and high-exposure-risk groups, including household or recent TB contacts (119), miners (124, 134), and prisoners (137). Certain known TB risk factors have not yet been studied in association with subclinical TB; these factors include immunosuppressive medications (e.g., tumor necrosis factor alpha [TNF-α] inhibitors or glucocorticoids) and residential or occupational exposure (e.g., health care workers). Further studies of these groups would presumably identify an elevated risk for subclinical TB. As better biomarker-based assays become available, researchers can prospectively monitor high-risk individuals to better describe the epidemiology of incipient and subclinical TB.

CLINICAL COURSE AND TRANSMISSIBILITY OF INCIPIENT AND SUBCLINICAL TB

Clinical Course

The clinical course of incipient and subclinical TB disease is driven by a complex interaction between pathogen and host defenses. As a result, the duration and trajectory of these disease states can differ substantially across patients (Fig. 1). It is possible that the highest-risk individuals (e.g., those with advanced HIV) progress rapidly from subclinical TB to active TB, whereas those at a more moderate risk remain in a protracted or cyclic disease state. Immunosuppressive conditions are important risk factors for progression and include less-prevalent but higher-risk conditions, such as HIV infection and TNF-α blockade, and more-common but lower-risk conditions, including malnutrition and diabetes mellitus (156, 157). The time since initial exposure is a risk factor for progression; for instance, reactivation of TB is highest in the first 2 to 5 years following infection (158). Importantly, while progression from latent to incipient, subclinical, and symptomatic TB disease is sometimes a unidirectional process, regression of disease may also occur (159).

Currently, little is known about the duration of incipient or subclinical TB disease states. Given this variable natural history, the time from initial infection to the development of incipient or subclinical TB may range from weeks to a lifetime. The duration of each disease phase is similarly heterogeneous, and a window period for incipient or subclinical TB disease may last from months to years. National prevalence surveys suggest that the duration of subclinical TB, as estimated by the prevalence-incidence ratio, is at least as long as that of symptomatic disease (115). However, observational studies in settings where HIV is endemic suggest that people with subclinical TB can quickly progress to active TB disease within weeks to a few months (119–122, 124).

The relationship between clinical symptoms and underlying disease burden may not be entirely proportional. To the extent that smear positivity measures bacillary burden in the lungs, subclinical TB is generally more likely to be smear negative than symptomatic TB, as suggested by national prevalence surveys (115) and observational studies in HIV-positive cohorts (118, 119, 126). However, since subclinical TB is a dynamic state, transitions from smear-negative to smear-positive disease or the development of new radiographic abnormalities as individuals progress from subclinical to symptomatic TB disease may occur (123, 124). Although the burden of *M. tuberculosis* in the lungs may correlate loosely with the presence of symptoms, extrapulmonary or disseminated TB in immunocompromised individuals is often asymptomatic, rapidly progressive, and fatal. Postmortem studies highlight the frequency of extrapulmonary disease in high-HIV-prevalence settings (140, 160, 161). Thus, heterogeneity in clinical presentation and diagnostic findings must be interpreted within the context of differ-

ing patterns of progression through incipient and subclinical TB as well as differing levels of host immune status.

Transmissibility

One of the key concerns is that persons who persistently or intermittently carry lower bacillary burdens in their lungs, as might occur with subclinical TB, may be important sources of transmission in the community (162). Transmission of TB can be considered to be a function of the degree of infectiousness, the duration of infectiousness, and the availability of susceptible contacts (163). Most models of TB transmission have not included a subclinical TB stage, which may constitute half of the average total duration of active TB prior to treatment. However, people with subclinical TB may likely be missed by current TB control efforts, especially in settings where TB culture is not included in intensified case-finding efforts. In a recent mathematical model where subclinical disease was included, active detection of 5% of prevalent TB cases had a substantially greater impact than a 20% increase in rates of passive case detection on diagnosis and mortality at 10 years in most epidemiological settings (163). More clinical studies are needed to fully characterize the contribution of subclinical TB disease to *M. tuberculosis* transmission.

SCREENING AND DIAGNOSTIC APPROACH

Until recently, screening and diagnostic tools have not been prioritized for either incipient or subclinical TB. Currently, there are no approved diagnostic tests for incipient TB, although several novel biomarkers are under investigation (108, 114). For diagnosing subclinical TB, conventional tools, including chest radiography, sputum smear microscopy, culture, and Xpert MTB/RIF (Cepheid Inc.), may be used with various utilities (4). Overall, better diagnostics for incipient and subclinical TB (2, 163–165), which could allow treatment of individuals before they become symptomatic and infectious, may be required to make significant progress for the WHO's "End TB Strategy" (166).

Current Approaches and Incremental Improvements for Incipient Tuberculosis

Tuberculin skin testing (TST) and IGRAs have be used to identify individuals with a persistent immune response to TB antigens. While these tests have well-described limitations (167–170), their biggest problem is that they poorly predict who is at greatest risk for disease progression (171, 172). In countries with a high burden of TB, a large proportion of the general population may be infected with *M. tuberculosis*, but few adults will progress to active TB disease within their lifetime. The TST and IGRAs are not specific for disease progression, and providing universal LTBI treatment is often impractical for national TB programs (173–176). Several newly developed tests for latent infection represent incremental improvements but appear to suffer from the same major shortcomings of not accurately predicting disease progression (177–181). One new assay, QuantiFERON TB Gold Plus (Qiagen), includes additional peptides designed to stimulate both $CD4^+$ and $CD8^+$ T cells. Early studies have demonstrated improved sensitivity over the traditional QuantiFERON-TB Gold in-tube assay and the potential to serve as a proxy for recent infection (178, 182–184). The WHO recently articulated the minimal and optimal characteristics for a target product profile of a test to predict progression from incipient TB infection to active TB disease (185).

Since incipient TB exists on a spectrum of TB progression, defining immunologically distinct characteristics has been challenging. Recent research on host transcriptional changes has relied on the measurement of immune markers and responses as a surrogate for TB disease burden. An early microarray identified innate antimicrobial genes that differentiated TB patients from LTBI patients (186). Independent blood-based genome-wide transcriptional profiles for patients in high-burden settings have revealed additional gene signatures, few of which have been mined for the enrichment of molecular pathways (69, 71, 187–189). In a whole-transcriptome approach, a 393-gene signature was derived by using a "molecular distance to health" composite score

that incorporated the severity of radiographical disease (68). The more recent Adolescent Cohort Study, performed in South African adolescents with LTBI, highlighted a 16-gene signature that could identify subjects as early as 18 months prior to the development of active TB (67, 114). The CORTIS study, a prospective validation trial to identify patients with incipient TB and guide preventive therapy using the same 16-gene signature, is under way (190, 191). Further improvements in simplifying the host RNA signature to a 6-gene set may also be possible (Thomas Scriba, personal communication), but the practicality of implementing this approach may remain challenging.

Current Approaches and Incremental Improvements for Subclinical Tuberculosis

In principle, tools used to diagnose active TB can also detect subclinical TB, although their sensitivity may be lower (192, 193). Since persons with subclinical TB do not have a symptomatic cough, they may be unable to produce quality sputum specimens, so novel assays may be necessary for use on non-sputum-based specimen types (194). Traditional diagnostic tests to confirm active TB disease include sputum smear microscopy, which has low diagnostic sensitivity (195), and mycobacterial culture, which has major infrastructure requirements and takes weeks to yield results (196). Xpert MTB/RIF, a nucleic acid amplification assay, has higher sensitivity than smear microscopy and important advantages over culture but has limited sensitivity to detect early or paucibacillary disease (197, 198). The next-generation Xpert MTB/RIF Ultra assay has improved sensitivity (199), but its ability to detect subclinical TB is unknown and still requires a sputum sample for the diagnosis of pulmonary TB. Several non-sputum-based approaches are currently under development for diagnosing active TB in both adults and children, which include *M. tuberculosis* nucleic acid detection in oral mucosa and stool (200, 201) as well the as detection of *M. tuberculosis* proteins and metabolites in urine (202). The most developed non-sputum-based assay detects urinary lipoarabinomannan (LAM) (203) and is indicated for use in severely immunocompromised HIV-infected hospitalized patients (204). However, the test is only moderately sensitive ($\sim$60%) among HIV positive adults with CD4 counts of $<$100 cells/mm^3 and has lower sensitivity in patients with CD4 counts of $>$100 cells/mm^3. Urinary LAM has been shown to detect subclinical TB in 25% (7/28) of HIV-infected ambulatory patients in South Africa (205). Despite these limitations, there is the potential to improve diagnostic sensitivity in next-generation urinary LAM assays (206–209).

Transformational Approaches and Remaining Challenges for Diagnostics

Transformational and novel approaches may be necessary to overcome limitations in diagnosing incipient or subclinical TB. In particular for incipient TB, a high-sensitivity test may be achieved by measuring the host immune response, as opposed to direct pathogen detection (210, 211). Several systems biology "omics"-based diagnostic approaches appear promising (212, 213). The most advanced approach has been peripheral blood host RNA-based signatures, which show the potential to differentiate the entire spectrum of TB (67) and may be suitable for the detection of incipient and subclinical TB. Multiple RNA signatures have been reported (69, 71, 214–217), only some of which have focused on incipient or subclinical TB. Recently, a meta-analysis of RNA data sets identified a 3-gene signature for differentiating TB from other respiratory diseases (218) and may be useful for detecting incipient TB (Purvesh Khatri, personal communication). Other novel approaches investigated for incipient TB, including cell activation and differentiation markers, cytokine levels in blood, and antigen-specific T cells, have also been described (219, 220).

While a more comprehensive approach may be promising, the eventual development, validation, and implementation of a complicated assay would be challenging. Translating a host RNA signature into a simple, affordable, and globally applicable test poses formidable technical challenges (221). Host-based signatures typically do not require simply differentiating "signal from noise" (i.e., a qualitative/binary assessment of the presence versus the absence of a pathogen marker) but instead require a precise

quantification of multiple biomarkers. Furthermore, validating possible diagnostic tests will pose unique challenges for tests targeting subclinical or incipient TB due to the lack of a good reference standard as well as requirements for sample size and longitudinal follow-up (185). Ensuring broadly generalizable accuracy estimates will also be difficult since host response signatures will likely vary depending on a multitude of factors, including age, ethnicity, medical comorbidities, the local environment, and various exposures to pathogens and immunological stressors (222, 223). Validation across a wide range of populations will be critical, but high levels of participant dropout can occur in the cascade of care for active TB (224, 225). Finally, many practical implementation questions will also need to be resolved, and additional work is needed on evaluating combinatorial approaches and complex algorithms.

TREATMENT AND PREVENTION OF INCIPIENT AND SUBCLINICAL TUBERCULOSIS

The basic principles underpinning treatment of TB infection and disease also apply to both incipient and subclinical TB (12). These include the microbiological burden (inferred from tests of bacterial load); disease extent (inferred from radiological studies); testing of susceptibility to major first- and second-line drugs; the site of disease for drug penetration; the risk of adverse events and medication side effects, including hepatotoxicity; and prognostic features and comorbidities, such as diabetes and HIV coinfection, with an increased risk of drug-drug interactions. Several of these factors may influence the drug-specific selection, dose, or duration of therapy for incipient and subclinical TB. All patients with suspected incipient or subclinical TB should have a comprehensive clinical evaluation for other comorbidities, including HIV.

Incipient Tuberculosis

Although pathophysiologically characterized, incipient TB has not previously had a clearly defined diagnostic definition, which has precluded the evaluation of a specific treatment regimen for a defined subgroup. However, incipient TB remains a critically important entity to diagnose, as preventing progression to active TB will optimize the use of health care resources. Although speculative, the conventional regimens for LTBI, which include 6 to 12 months of isoniazid, 3 months of a combination of rifamycin and isoniazid, or 3 to 4 months of a rifamycin alone, may be effective for incipient TB, assuming a relatively low burden of disease. However, newer, existing, and repurposed drugs (bedaquiline, delamanid, linezolid, likely sutezolid in the future, and fluoroquinolones) are now being used in clinical practice to treat drug-resistant TB (226, 227). Once issues of toxicity, teratogenicity, and effectiveness are clarified, newer and repurposed drugs offer the prospect of simplifying and shortening regimens for incipient TB of any susceptibility profile in the future. Another treatment option is providing a high dose of rifamycins, which have the potential to shorten the duration of conventional treatment for active TB and are well tolerated (228). Clinical trials are already in progress to evaluate the utility of different drugs, including delamanid and fluoroquinolones, for the treatment of multidrug-resistant LTBI (229). If successful, then they may also be appropriate for treating incipient TB. Future, alternative approaches for the treatment of incipient TB might include therapeutic vaccines, host-directed therapies, or combining immunosuppressive agents and anti-TB drugs (230). Future clinical trials will also need to address the issue of duration of therapy for incipient TB. In countries where TB is endemic, the WHO recommends treating presumed LTBI in HIV-infected persons with isoniazid for 36 months (231), which may be a starting point for incipient TB.

Subclinical Tuberculosis

Treatment of subclinical TB should include concurrent HIV testing and, where possible, obtaining a biological sample for drug susceptibility testing. The presence of HIV coinfection will impact the choice of antiretroviral therapy and raise considerations related to immune reconstitution inflammatory syndrome, while regimens for the treatment of drug-resistant TB will depend on the susceptibility profile of the *M. tuberculosis* isolate. However, in general, treatment for subclinical TB should be identical

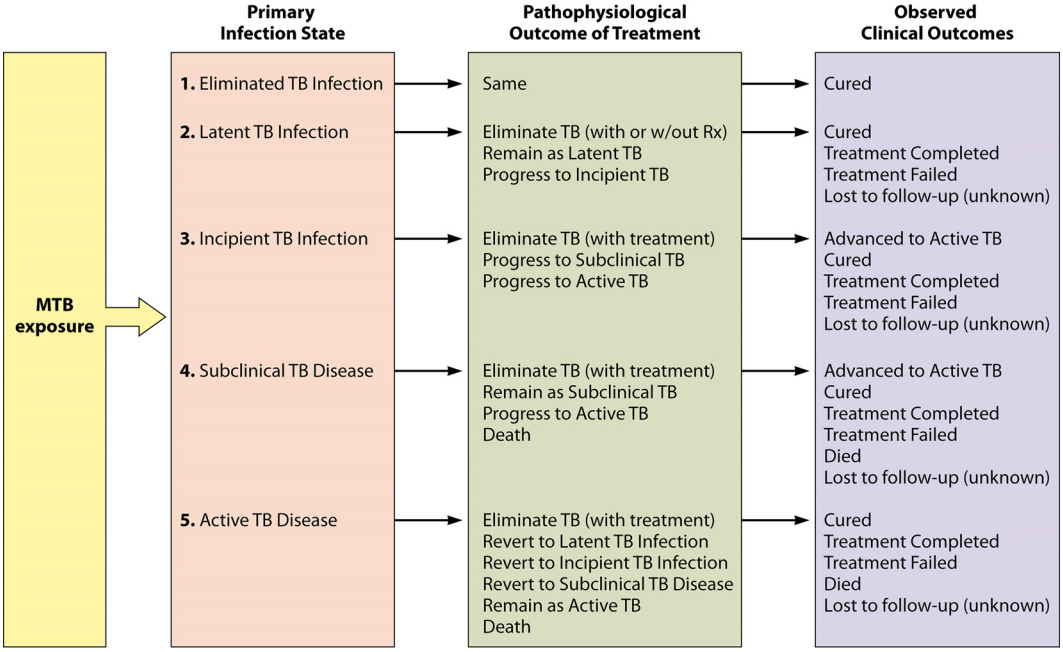

FIG 2 Primary and secondary disease states for the five categorical states of TB. Clinical outcomes following treatment are variable and depend on the respective pathophysiological outcomes. MTB, *M. tuberculosis*.

to that of conventional active TB disease while taking into account comorbidities, potential drug-drug interactions, and other considerations described above. Once results from clinical studies that are evaluating newer and repurposed drugs become available, additional trials may need to be initiated to evaluate the possibility for shortening the duration of treatment for drug-sensitive subclinical TB, based on disease extent and bacterial burden. One clinical trial is currently evaluating the utility of a short 4-month treatment course, compared to a standard 6-month regimen, based on bacterial burden and the radiological extent of disease (232). While ongoing studies are directed at patients with active TB disease, future studies will need to validate or improve treatment options for patients with subclinical TB.

Isoniazid Preventive Therapy

Persons with incipient or subclinical TB are sometimes identified as having LTBI and may be started on isoniazid preventive therapy (IPT). While this practice raises concerns for the emergence of isoniazid resistance and the potential for ongoing disease transmission (233, 234), IPT-driven drug resistance, despite decades of use, has not been confirmed at the population level (235). In one study, treatment was switched to a four-drug regimen upon the recognition of subclinical TB, and participants had a good response (123). Furthermore, a comparison of recently IPT-treated and -untreated individuals with subclinical disease did not show differences in the proportions of individuals who subsequently developed active TB (119). Although evidence is limited, observational studies have not suggested a benefit of IPT on the clinical progression or outcomes of subclinical TB disease (155). Despite a lack of evidence, it will be essential to first exclude subclinical TB when considering treatment for LTBI. In contrast, preventive regimens are appropriate in the setting of incipient TB.

Treatment Outcomes

Responses to treatment for each stage of disease are variable (Fig. 2). Clinical outcomes are rarely recorded for the treatment of LTBI, and the appropriate treatment course for incipient or subclinical TB remains uncertain. Currently, treatment outcomes for active TB are often reported simply as treatment success (i.e., completion of a full course of treatment and resolution of symptoms) versus failure (persistent disease), loss

TABLE 4 Priority research questions for incipient and subclinical tuberculosis

Question
Pathogenesis and immune response
What triggers/prevents progression from incipient or subclinical to active TB disease?
How do different strains of *M. tuberculosis* alter proliferation and progression to active TB?
Can the immune system be harnessed to stop *M. tuberculosis* in the incipient or subclinical stage?
Epidemiology
How does the prevalence of incipient and subclinical TB vary across populations?
Are there host genetic factors that may increase the risk of incipient or subclinical TB?
Clinical course and transmissibility
How common is a waxing/waning picture of adults with subclinical and active TB?
What are the risk factors for progression of disease through incipient and subclinical TB?
How transmissible is TB in the incipient or subclinical disease stage?
Should contacts of a patient with incipient or subclinical disease be evaluated for TB?
Screening and diagnostic approach
What are the optimal screening and diagnostics tests for incipient TB?
What are the optimal screening and diagnostics tests for subclinical TB?
What is the recommended screening frequency for either stage in high-TB-burden regions?
Treatment and prevention
Should incipient TB be treated with isoniazid monotherapy or another regimen, and for how long?
Should subclinical TB be treated with monotherapy, 4-drug therapy, or a new drug combination?

to follow-up, or death (13). Cure is sometimes confirmed microbiologically, but even microbiological cure does not necessarily equate to pathophysiological cure (i.e., the absence of viable bacilli in the host). One study that profiled patients from multiple cohorts infected with drug-sensitive *M. tuberculosis* after 6 months of anti-TB therapy found that a substantial number of people still showed ongoing inflammation in the lung by FDG PET/CT and that some developed new or intensified lesions during the course of therapy (41, 236). Thus, "treatment success" as recorded clinically may correspond with regression to an earlier stage in the pathophysiological spectrum of disease.

RESEARCH NEEDS AND PRIORITIES

Earlier diagnosis and prompt therapy for incipient or subclinical TB may be critical to preventing TB disease progression and transmission of *M. tuberculosis* bacilli. Since both incipient TB and subclinical TB are relatively new concepts, many clinical, translational, and basic science research questions remain. In Table 4, we list some of the key research needs and priorities for each of the domains presented in this review. This list is not meant to be comprehensive but rather is meant to present some prior research questions for the current field.

Several basic science questions pertain to the complex host-pathogen interactions that may either promote or halt the progression of infection from an incipient or subclinical TB stage to active TB disease. Additional research is needed to determine whether the powers of the host immune system may be harnessed to existing or novel host-directed therapies to stop *M. tuberculosis* in the incipient or subclinical stage. To improve the understanding of TB epidemiology, more research is needed on the underlying prevalence and incidence of both incipient and subclinical TB across various high- and low-risk populations. Many research questions also exist for the clinical course and transmissibility of incipient and subclinical TB. Some of these questions include the general pattern of disease progression and the associated risk factors, while the apparent transmissibility of subclinical TB is largely unknown but clearly important for adequate infection control and public health responses. Finally, the optimal diag-

nostic modalities and treatment of incipient and subclinical TB have been largely underexplored but are quickly becoming important priority topics.

CONCLUSIONS

The recognition of incipient and subclinical TB as distinct states between latent and active TB along the clinical disease spectrum provides opportunities for an improved understanding of disease dynamics as well as the development of diagnostic and therapeutic interventions to prevent progression to active TB disease and transmission of active TB bacilli. However, this revised understanding of TB may not currently change the therapy directed to individual patients, mainly because of the major limitations with the available diagnostic methods and tools. In this review, we have highlighted the current knowledge of incipient and subclinical TB and offered opportunities and priorities for further exploration.

In 2018, the United Nations General Assembly will hold its first high-level meeting on *M. tuberculosis* and the global TB epidemic, roughly 136 years after the initial discovery of a causative agent for TB. The meeting will likely highlight the need for more innovative solutions to an ancient disease, which should include priority research questions around incipient and subclinical TB.

Through the development of a better understanding of the clinical pathogenic spectrum of TB infection and disease, we may be able to devise more advanced and specific solutions to the TB epidemic. By synthesizing the expanding knowledge and addressing existing research gaps for both incipient and subclinical TB, the research and public health communities may move forward together on developing sustainable solutions to end the TB epidemic.

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