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VIEWPOINT

Challenges in TB research

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Tuberculosis (TB) is an infectious disease bedeviled by complexity. This poses myriad challenges for a research ecosystem organized around specialist host- and/or pathogen-focused thrusts. Here, we highlight the key challenges and their implications for developing new tools to control TB.

Tuberculosis (TB) is consistently among the leading annual causes of infectious deaths globally, claiming at least 1.66 million lives in 2021 (WHO, 2022). With hard-earned gains in TB control having been cruelly erased by the COVID-19 pandemic (Chakaya et al., 2021), indications are that TB will shortly regain the position of top infectious killer globally. On the face of it, the staggering toll of TB is hard to reconcile with the availability of an effective neonatal vaccine and frontline drug regimens. However, the existing tools have proven inadequate in reducing the burden of TB and halting the emergence of multidrug resistance. To achieve the aspirational goals of the WHO's End TB Strategy, there is an urgent need to intensify research on this age-old disease that feeds on socioeconomic inequalities and is fraught with manifold challenges. Here, we reflect on the complexity of TB and how this undermines our ability to intervene, in part because it encourages the diversification of researchers into discrete thrusts that focus on one aspect of the disease, so that advances (there have been many) are slow to be assimilated into a framework that necessarily incorporates the aetiologic agent, *Mycobacterium tuberculosis* (Mtb), its human host, and the environment (Fig. 1). Similarly, developing models to reflect these multi-pronged interactions has been challenging, and is exacerbated by the difficulties in aligning clinical disease definitions with the molecular, microbiological, genetic, and social complexity which combine to drive Mtb exposure to TB disease.

Breathing as a risk factor to expel and inhale the pathogen

TB is caused by members of the Mtb complex. An obligate human pathogen, Mtb is transmitted via droplet bioaerosols that are small enough (0.5–5 µm) to remain suspended in air for extended periods of time and to access lung alveoli once inhaled. Identifying how, when, and by whom Mtb is aerosolized is key to interrupting transmission. Historically, cough—a common symptom of TB—was thought to be the dominant source of infectious bioaerosol. This thinking informed strategies that sought to interrupt transmission by finding and treating individuals with active, symptomatic disease. However, new insights are challenging some of the assumptions underlying this strategy. National prevalence surveys in countries across Africa and Asia have reported high proportions of bacteriologically confirmed TB among asymptomatic individuals, with estimates indicating ∼50% of TB is subclinical (Frascella et al., 2021). An added complication is that many individuals with active TB (especially HIV-positive and pediatric patients) are unable to expectorate sputum, which precludes obtaining the definitive diagnosis required to initiate treatment. In a new study on the aerobiology of TB, symptomatic TB patients were shown to release Mtb aerosols in the 0.5–5 µm size range not only by cough, but also by tidal breathing at rates which suggest that tidal breathing might be a significant contributor to transmission by active TB cases (Dinkele et al., 2022). While Mtb aerosol release has yet to be quantified in asymptomatic or subclinical TB, this group of individuals, who fall beneath the diagnostic threshold for active TB, must be considered as possible contributors to transmission (Kendall et al., 2021). It is therefore imperative that research to develop new tools for identifying and treating all those who expel infectious organisms is prioritized.

Stealth and survival strategies of a Trojan horse with many guises

Mtb shares an evolutionary history with its human host that spans thousands of years, and the bacillus has evolved to infect, survive, and replicate in human phagocytic cells. Once inside primary alveolar phagocytes, Mtb is adept at subverting and evading effective anti-microbial immune mechanisms, exploiting immune cell trafficking to spread within the lungs and via hematogenous routes to reach lymphoid tissues and virtually any organ in the body. The paucibacillary nature of the early stages of disease poses major challenges for the direct detection and quantification of Mtb in infected individuals; moreover, the relationship between Mtb number and extent of pathology (and, consequently, disease

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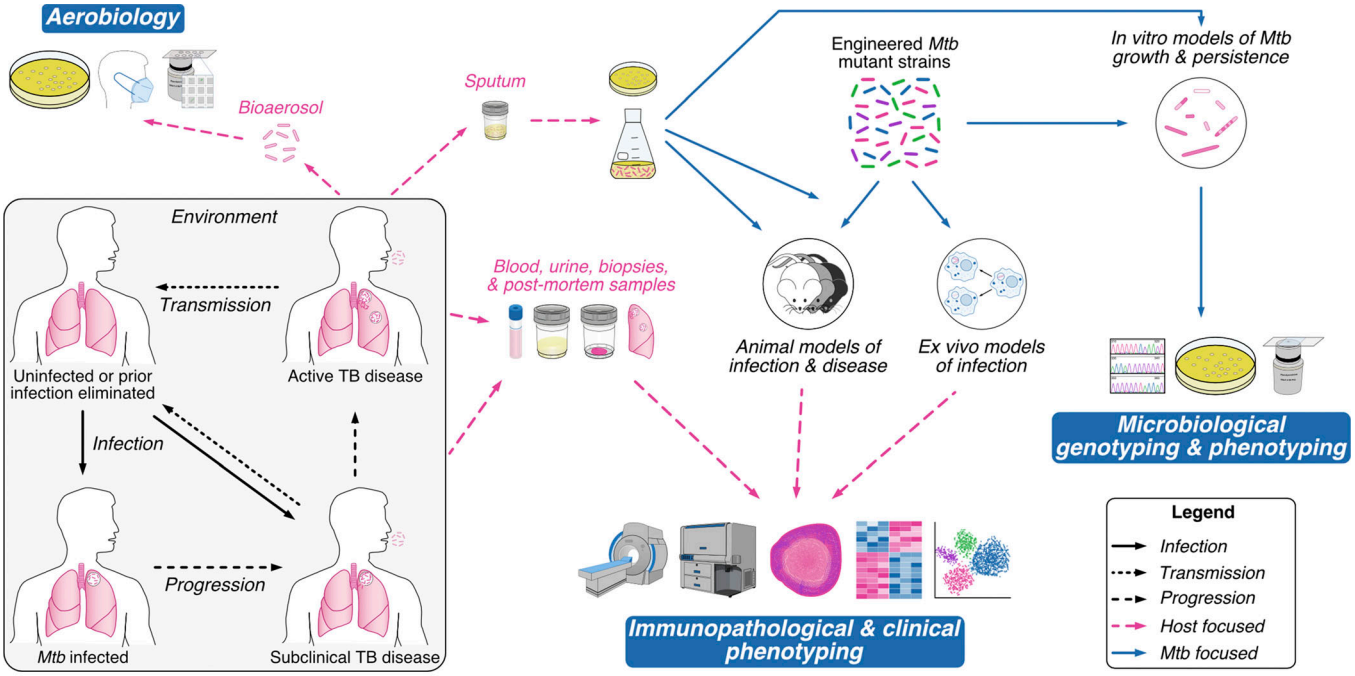

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Figure 1. **The TB research ecosystem.** TB is a disease fraught with complexity at every level, from the transmission, infection, and disease cycle of the host to the diverse phenotypic and genotypic variation of the pathogen. Recapitulating this complexity in a single experimental system has proven challenging, an unintended consequence being the siloing of research thrusts focusing primarily on the host (pink arrows) or the pathogen (blue arrows), seldom both, and very rarely incorporating the environments in which natural infection and disease occur.

outcome) remains uncertain (Lin et al., 2014). In early disease stages, the detection of Mtb infection is based instead on the presence of mycobacteria-specific T cells. However, persisting T cell memory responses in those with a prior, cleared infection remain positive, confounding the utility of "tests of Mtb infection." These challenges critically limit our ability to identify and study those with asymptomatic infection or subclinical disease and must be urgently addressed.

Survival of the Mtb bacillus is also enabled by genetic and phenotypic heterogeneity. Genomic diversity within and between lineages of the Mtb complex may impact the organism's transmissibility, virulence, intrinsic drug susceptibility, and propensity to acquire genetic drug resistance. Adding to this complexity, genetically clonal mycobacterial populations are characterized by phenotypic heterogeneity. This arises from asymmetric cell division, which imparts different phenotypic characteristics on daughter cells owing to unequal distribution of macromolecules and metabolites, and from a flexible metabolic repertoire that supports survival in multiple intra- and extracellular microenvironments. Heterogeneity complicates our understanding of disease etiology and confounds attempts at treatment shortening (Chung et al., 2022). Recognizing this capacity for adaptation, approaches to TB drug discovery are incorporating models that reproduce relevant features of natural Mtb infection (Aldridge et al., 2021; Dartois and Rubin, 2022).

Grappling with intra- and inter-host heterogeneity

The complexity extends beyond the causative organism to disease pathology, which is characterized by remarkable within- and between-host variability (Cadena et al., 2017). It was recognized decades ago that TB lesions and pathology within a single individual can be highly diverse, even divergent. The application of advanced radiological techniques in studying the response of TB patients to curative therapy has shown that, while some lesions regress, others within the same individual progress (Malherbe et al., 2016). Other studies have elegantly revealed the intra-host heterogeneity in granuloma pathology, inflammation, and bacillary load within lungs and lymph nodes of Mtb-infected non-human primates (Martin et al., 2017). Inter-individual variability among Mtb-infected people is similarly diverse; TB is now best understood as a spectrum that includes those who resist infection despite prolonged exposure (resisters), people with long-standing asymptomatic infection, and those with minimal, subclinical, or symptomatic active disease (Drain et al., 2018). Disease progression typically follows diverse trajectories, including spontaneous regression, even in those with infectious, active disease. This heterogeneity is difficult to ascertain with existing tools but its implications are profound, and range from complicating efforts to understand TB pathogenesis to impacting on the ability of drugs to reach bacteria located in diverse—and potentially dynamic—host environments (Dartois and Rubin, 2022).

Inducing immunity against Mtb

The development of vaccines for TB is beset by myriad challenges. Mtb is a slow-growing organism with a protracted pathogenesis. Following exposure and infection, progression in those who develop TB disease

occurs within 2–3 yr. Consequently, clinical trials to assess vaccine efficacy against TB disease must be very long, typically 5–7 yr. Despite this, recent proof-of-concept trials, one of Bacille Calmette Guérin revaccination in adolescents (Nemes et al., 2018) and the other of the subunit vaccine M72:AS01$_E$ in adults with evidence of prior infection (Tait et al., 2019), reported good efficacy, prompting follow-up trials that are underway. Vaccine development could be dramatically accelerated by an accurate and validated correlate of vaccine protection, a response that predicts protective immunity against Mtb and can be measured weeks after vaccination. Despite years of research toward correlate of vaccine protection discovery, such markers remain elusive. However, a major international effort using samples from the recent proof-of-concept trials is underway (Nemes et al., 2022).

Many other questions pertinent to vaccine development remain inadequately addressed, especially if the goal is to exceed the 50% efficacy observed with M72:AS01$_E$ (Tait et al., 2019). For example, which of the thousands of Mtb proteins is/are ideal for use as vaccine antigen; can adjuvant science modulate immune responses to achieve higher efficacy; can vaccine-induced responses promote immunopathology; and will new TB vaccines be enthusiastically adopted by communities at risk of TB?

Addressing TB as a systemic challenge

Mirroring this complexity, the TB field has organized around specialist research thrusts that, albeit delivering important advances, have struggled to cohere in addressing TB as a systemic challenge. Reframing the TB narrative while collapsing barriers among TB researchers will be critical if transformative progress is to be made toward ending the TB pandemic. However, TB is a barometer of socioeconomic and health disparities: it disproportionately affects poor and vulnerable people who are at increased risk of Mtb infection and disease and have worse health outcomes. Many poverty-associated factors are known to increase risk of TB, including overcrowding, co-prevalent infections and diseases including HIV and diabetes, poor nutrition, substance abuse including smoking and alcohol, and exposure to indoor air pollution. While new tool development will require far greater resourcing for TB research, any hope for controlling TB with new and existing biomedical interventions will ultimately rest on the simultaneous implementation of programs for economic development and improved access to healthcare for vulnerable groups. This, in turn, requires political will on a global scale not yet seen. Lessons from the COVID-19 pandemic paint a bleak picture: We can and must do better for TB.

Disclosures: The authors declare no competing interests exist.

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