The Microbiome and Tuberculosis: Early Evidence for Cross Talk

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ABSTRACT Tuberculosis (TB) is an ancient infectious disease of humans that has been extensively studied both clinically and experimentally. Although susceptibility to Mycobacterium tuberculosis infection is clearly influenced by factors such as nutrition, immune status, and both mycobacterial and host genetics, the variable pathogenesis of TB in infected individuals remains poorly understood. During the past two decades, it has become clear that the microbiota—the trillion organisms that reside at mucosal surfaces within and on the body—can exert a major influence on disease outcome through its effects on host innate and adaptive immune function and metabolism. This new recognition of the potentially pleiotropic participation of the microbiome in immune responses has raised the possibility that the microbiota may influence M. tuberculosis infection and/or disease. Similarly, treatment of TB may alter the healthy steady-state composition and function of the microbiome, possibly affecting treatment outcome in addition to other host physiological parameters. Herein, we review emerging evidence for how the microbiota may influence the transition points in the life cycle of TB infection, including (i) resistance to initial infection, (ii) initial infection to latent tuberculosis (LTBI), (iii) LTBI to reactivated disease, and (iv) treatment to cure. A major goal of this review is to frame questions to guide future scientific and clinical studies in this largely unexplored but increasingly important area of TB research.

KEYWORDS antibiotics, microbiome, tuberculosis

Tuberculosis (TB) sickens more than 10 million people each year and kills 10 to 20% of them; TB is the leading cause of mortality by a single infectious agent (1) and ranks in the top 10 causes of death in the world. Mycobacterium tuberculosis is transmitted by aerosols from individuals with active TB. Although approximately one-third of the world’s population is latently infected (LTBI), only 5 to 10% develop active disease in their lifetime. Moreover, even in settings of high exposure, a sizable percentage of the population remains tuberculin skin test (TST) or interferon gamma (IFN-γ) release assay (IGRA) negative (2), suggesting that they have been able to resist infection despite likely M. tuberculosis exposure. Although drug-sensitive active TB can be cured with 6 months of daily antibiotic treatment, this long duration of therapy is frequently complicated by noncompliance, relapse, and the development of drug resistance. Often lost in the discussion of the long duration of therapy required to cure TB is the large heterogeneity in response to treatment. Six months is the shortest duration of therapy that will reliably cure >95% of subjects; however, often overlooked is the fact that >60 to 70% of subjects are cured by shorter-course regimens (3–5). Indeed, at present we have no way of predicting which patients need longer courses.
of treatment. Shorter regimens would be successful if we could identify biomarkers that allow those able to be cured in less than 6 months of therapy.

During the timeworn history of research on TB in humans, a number of underlying risk factors for the disease have been identified (6) that contribute to the total population attributable risk percentage (PAR%), a measure of the fraction of disease that can be accredited to a specific risk factor. Many of the risk factors directly or indirectly involve the immune system. These include HIV infection (>10 times the risk for LTBI to active disease transition) (7–9), genetic immunodeficiency (10), age (~10% of active TB disease cases) (1, 11), indoor air pollution (>20% of TB incidence) (12), malnutrition (>25% of TB incidence) (12), metabolic syndrome (13), the use of immunosuppressive drugs (14), and substance abuse, including alcohol (15) and smoking (16, 17). These PAR estimates are imprecise due to their underlying assumptions and inability to account for overlapping exposures; nevertheless, they are an effective public health tool to understand causes of disease and prioritize potential research and health interventions (18). Mechanistically, some of these risk factors involve well-defined immunological parameters, such as the requirements for CD4 T lymphocytes and the cytokines interleukin-12 (IL-12), IFN-γ (19), and tumor necrosis factor alpha (TNF-α) (20). However, millions of people acquire LTBI or get sick with active TB disease every year with no apparent immunologic deficiency, suggesting the presence of additional, as yet unidentified, risk factors to explain the full PAR%. In this review, we summarize recent evidence implicating the composition and function of the microbiome as an additional risk factor for *M. tuberculosis* infection and TB disease progression.

**MECHANISMS BY WHICH THE MICROBIOME MIGHT INFLUENCE TUBERCULOSIS BIOLOGY**

With the advent of high-throughput sequencing technologies, the last decade has seen an explosion of microbiome research in which the composition of the microbiota has been profiled in a wide variety of diseases. In most cases, although plausible, the causal link between these commensal alterations and specific disease states (21–24) has not been established. The microbiome might contribute to tuberculosis risk and disease (i) by determining interindividual differences in immune cell subsets or function that may influence tuberculosis susceptibility or response to therapy, either remotely from the intestinal microbiota or directly in the lung, (ii) by affecting drug absorption during tuberculosis treatment, and/or (iii) by producing antimicrobial or immune activating molecules that may influence *M. tuberculosis* growth directly (25) (Fig. 1). Here we review the newly emerging literature addressing whether the composition of the microbiota changes with TB disease status, influences susceptibility to infection, or affects the response to therapy. We have organized the discussion around the major stages of clinical TB, namely (i) initial *M. tuberculosis* infection, (ii) TB disease progression, (iii) response to antibiotic treatment, and (iv) posttherapy cure and reinfection.

**POTENTIAL INTERACTION POINTS OF THE MICROBIOTA WITHIN THE TB LIFE CYCLE**

**Resistance to initial infection.** Tuberculosis is transmitted by inhalation of droplet aerosols liberated from the lungs of active tuberculosis patients through coughing. With inhalation, successful infection of the host leads to LTBI, which is detectable by TST/IGRA. It is presumed that some exposed contacts who inhale the bacterium are able to eliminate the infection before the establishment of latency and the accompanying *M. tuberculosis*-specific T cell responses through which LTBI is diagnosed. The explanation for why these individuals who are directly exposed remain uninfected (TST−/IGRA−) is unclear. One possibility is that differences in the microbiota could influence the clearance of initial infection through innate immune mechanisms. Comparisons of wild-type and germfree mice have revealed differences in transcriptional profiles of innate lymphoid cells (26), myeloid cell development and function (27–29), and mucus layer formation (30). Indeed, a number of studies have demonstrated the
importance of the interaction between the microbiome, its metabolites, and the host innate immune system in maintaining organismal homeostasis, including immune tolerance and defense against pathogens (31, 32). In this regard, there are data indicating that certain commensal bacteria and their antimicrobial products can quantitatively influence the (initial) resistance to pathogens (e.g., vancomycin-resistant enterococcus, Clostridium difficile, and Salmonella enterica serovar Typhimurium [33–35]) via a variety of mechanisms, including niche competition (36) and bacteriolytic activity (37, 38). Nevertheless, data on the role of the microbiota in mediating initial resistance to TB infection are limited. Mice treated with broad-spectrum antibiotics were found in one study to be modestly but significantly more susceptible to aerosol M. tuberculosis challenge, particularly when bacterial burden was assessed at extrapulmonary sites (39). A more recent study utilized a mouse model of Helicobacter hepaticus in which infection with this commensal causes a defined change in the gut microbiota. The resulting dysbiosis led to an increase in bacterial burden following M. tuberculosis challenge (40). Although speculative at this time, such effects of the microbiota on initial host resistance could affect susceptibility to infection in exposed humans. This question could be approached by comparing the microbiome compositions of equivalently exposed IGRA+ and IGRA− household contacts of active TB cases and relating the differences seen to possible host protective functions.

**Progression from LTBI to active TB disease.** As previously noted, numerous mechanisms have been proposed to explain the emergence of active TB in individuals...
with LTBI. In several studies, the microbiota has been indirectly implicated as a factor in disease progression. In one report, it was shown that LTBI patients with *Helicobacter pylori* in their gut flora were 50% less likely to develop active TB disease (41). This observation is consistent with other studies demonstrating similar associations between the presence of certain intestinal bacteria and pulmonary susceptibility to the manifestations of experimental respiratory syncytial virus infection and pediatric asthma (42, 43).

A recent report presented evidence for a possible link between the transition from LTBI to active disease and the composition of the lung (as opposed to intestinal) microbiota in HIV-infected South Africans (44). This study demonstrated that within a cohort of HIV-infected patients on antiretroviral therapy (ART), higher concentrations of two short-chain fatty acids (SCFAs) in serum, propionate, and butyrate were associated with increased risk for active TB. The elevation in SCFAs correlated with a corresponding increase in the abundance of a number of anaerobic bacteria, including *Prevotella*, a genus known to produce these lipid molecules. The same SCFAs suppressed *in vitro* production of IFN-γ and IL-17A by both polyclonal-stimulated T cells and TB antigen-stimulated peripheral blood mononuclear cells (PBMCs). Together, these results suggested that in some patients, ART treatment is associated with increased levels of certain pulmonary anaerobes that in turn result in increased SCFA production and suppressed T cell effector function. A broader implication of this study is that the metabolic activity of the microbiota at mucosal surfaces may be an important risk factor for the development of active TB. In this regard, indole-3-propionic acid (IPA), a metabolite produced by *Clostridium sporogenes* (45) and other gut commensals, was recently shown to exhibit antitubercular activity *in vitro* and at extrapulmonary sites in a murine experimental model (46). The mechanism underlying this antimicrobial effect remains to be elucidated.

Another plausible mechanism by which commensal metabolites may influence TB progression is through their role in stimulating innate T cell subsets through the major histocompatibility complex (MHC) class I-like proteins CD1 (47) and MR1 (48–50). These MHC-like proteins are restriction elements for the activation of invariant natural killer T (iNKT) cells (51), germ line-encoded mycolyl lipid-reactive (GEM) T cells (52), and mucosa-associated invariant T (MAIT) cells (48–50), all of which have been speculated to be involved in host resistance to *M. tuberculosis*. MAIT cells are of particular relevance since they proliferate in response to MR1-bound riboflavin biosynthetic intermediates, which can be synthesized by *M. tuberculosis* and are enriched at the sites of infection (53, 54). Interestingly, MAIT cells are absent in germfree mice (48), suggesting that their development and function may be influenced by the microbiota, possibly through the production of MR1-binding molecules. It is therefore plausible, although as yet unexamined, that differences in microbiome composition could influence tuberculosis progression through an effect on the abundance or function of these innate, bacterium-reactive T cell subsets.

Prospective studies that follow the development of active disease in cohorts of LTBI patients are needed to directly identify associations between microbiota composition and LTBI progression in humans. This type of analysis has been employed to identify host transcriptional profiles that correlate with, as well as predict, active TB in LTBI individuals (55). Indeed, correlations between blood transcriptional signatures and microbiota composition may exist that would be of important diagnostic as well as mechanistic significance.

**Active TB versus no *M. tuberculosis* infection.** Two studies have examined changes in the microbiota that occur during the course of active TB in mice. Inbred specific-pathogen-free (SPF) mice, in which the intestinal microbiota is relatively uniform between animals, revealed only minor alterations in taxa as a consequence of infection with aerosol *M. tuberculosis*. These changes occurred largely in the relative abundance of the order *Clostridiales* (56, 57).
A number of microbiome studies in humans demonstrate interindividual (as well as geographic) differences in the steady-state microbiome with respect to *M. tuberculosis* infection or TB disease. In these reports, the microbiota was analyzed in the stool, sputum, or bronchoalveolar lavage (BAL) fluid of diseased patients. In the case of the intestinal microbiota, a recent study found increased diversity and levels of *Actinobacteria* and *Proteobacteria* in patients with recurrent TB and decreased levels of *Prevotella*, as well as members of the order *Clostridiales*, in both new and recurrent TB patients in comparison to healthy individuals (58).

Current information on changes in the lung (as opposed to intestinal) microbiome induced by active *M. tuberculosis* infection is based on several studies on sputum samples (59–62) and one study employing BAL fluid (63). Such analyses of the pulmonary flora are inherently more complicated than those involving the fecal microbiota due to practical limitations in obtaining material free of oral bacterial contamination, as well as the difficulty of enrolling healthy controls for BAL fluid extraction, an invasive procedure. Moreover, the lung microbiome because of its lesser abundant biomass and transient nature (64) makes its accurate analysis more challenging. Nevertheless, distinct changes in the diversity and composition of the lung microbiota based on sputum have been associated with new *M. tuberculosis* infection, recurrent TB disease, and treatment failures in humans (60), although it is difficult to discern a common pattern between the different studies, with a possible exception of increases in the common lung bacteria *Streptococcus* and *Pseudomonas*, which appear in multiple reports (65). Interestingly, although one might predict a decrease in diversity due to overcrowding of the pulmonary niche by the mycobacteria themselves, such a reduction has not been routinely observed. In addition to the above-mentioned limitations in performing human studies, our understanding of the interaction of *M. tuberculosis* infection and TB disease with the pulmonary microbiome has suffered from the absence of data from animal models, in part due to the lower abundance of microbiota at that tissue site. Current research on experimental *M. tuberculosis* infection in nonhuman primates presents an opportunity to address this shortcoming.

**Effect of TB antibiotic treatment on the microbiota.** Antibiotics are a major cause of microbiota perturbation since these molecules are designed to specifically and/or broadly kill bacteria (66). Antibiotic exposure early in life is a risk factor for the development of asthma, diabetes, and weight gain later in life (67, 68). Adults taking various antibiotics can develop antibiotic-associated diarrhea (69, 70), show increased susceptibility to pathogen colonization (71, 72), and in certain cases show impaired responses to other immune-based therapies, such as checkpoint blockade for malignancy (73–75). Each of these has been linked to alterations of the intestinal microbiota, where different classes of antibiotics with distinct mechanisms of action have unique effects on the composition of the commensal flora (66).

As noted above, treatment of drug-susceptible TB requires multiple daily administrations of oral antibiotics for a duration of at least 6 months according to World Health Organization guidelines (76). Millions of people receive these drugs every year, making TB chemotherapy one of the most widely administered treatment interventions, as well as one of the longest-duration antibiotic regimens utilized globally. Of the four first-line antibiotics used in TB treatment, only rifampin (R/RIF) has a broad-spectrum activity against a wide range of Gram-positive and Gram-negative bacteria. Isoniazid (I/INH), pyrazinamide (Z/PZA), and ethambutol (E/EMB) specifically target mycobacterial species, with isoniazid and pyrazinamide being prodrugs that need to be activated by mycobacterium-specific enzymes, which then inhibit or require for function mycobacterium-specific targets (77–80). Due to this mycobacterial specificity, the effects of antituberculosis treatment on intestinal or pulmonary microbiome composition are not predictable and, until recently, were unknown.

**Acute effects of TB treatment on the microbiota.** Recent studies in mice and TB patients have examined the effects of TB treatment on the microbiome (Table 1). These studies have shown that conventional TB antibiotic therapy causes a defined and
persistent dysbiosis in the intestinal microbiota, which somewhat unexpectedly has marked similarities in terms of the taxa altered in the two host species (57, 81) (Fig. 2).

The first surprising observation is that isoniazid-rifampin-pyrazinamide (ethambutol) [HRZ(E)] treatment has minimal effects on the diversity of the intestinal microbiome, a unitary metric of species number (richness) and distribution (evenness). In mice, there is only a transient decrease in diversity (57), while in humans who have been in treatment for at least 3 months, no significant change in diversity was observed (81). Thus, diversity during chronic HRZ(E) treatment may not be a good metric of mycobacterial drug effects.

In terms of the specific taxa affected by HRZ(E) treatment, both mice and humans have shown strikingly similar effects on the order Clostridiales of the phylum Firmicutes. Clostridia are important players of gut homeostasis, barrier function, and metabolism, particularly via their production of SCFAs (82). Among the members of the Bacteroidetes phylum, Bacteroides species are decreased with TB antibiotic treatment, whereas Prevotellaceae are increased—a shift that has also been associated with a protein versus carbohydrate-enriched diet (83). Among the prominent increases in microbiota ob-

| Antibiotic(s)* | Effect on intestinal microbiota | Reference |
|---------------|-------------------------------|-----------|
| HRZ (mice)    | Decreases in Acetivibrio, Robinsoniella, Alkaliphilus, Stomatobaculum, Butyricoccus, Acetanaerobacterium, Tyzzerella, Ruminococcus, and Peptococcus and increase in Erysipelatoclostridium | 57        |
| Post-HRZ (mice)| Decrease in Lactobacillus and increase in Barnesiella, Porphyromonas, Paraprevotella, Parasutterella, and Desulfovibrio and Actinobacteria genera | 57        |
| HRZE (humans) | Decrease in Lactobacillus, Coprococcus, Ruminococcus, and Bifidobacterium and increase in Erysipelatoclostridium, Fusobacterium, and Prevotella | 81        |
| HRZE (humans) | Decrease in Prevotella and Lachnospira | 58        |
| Post-HRZE (humans) | Decrease in Bacteroides and increase in Faecalibacterium, Eubacterium, and Ruminococcus | 81        |
| H alone       | Alterations in Barnesiella and certain Clostridium species | 57        |
| R alone       | Decrease in diversity and a number of Clostridium species | 57        |
| Z alone       | Alterations in Anaeroplasma and certain Clostridium species | 57        |

*Abbreviations: H, isoniazid; R, rifampin; Z, pyrazinamide; E, ethambutol.

FIG 2 Cladograms depicting the parallel effects of TB antibiotic treatment on the intestinal microbiomes of mice and humans. Two recent studies reported the effects of antituberculosis therapy, HRZ(E), on the gut flora of mice (57) and humans (81). A combined comparison of data from naive/healthy mice/humans versus mice administered HRZ or humans taking HRZE is shown, based on published (57, 81) as well as additional unpublished data. In both host species, in comparison to corresponding healthy untreated controls, members of the order Clostridiales were depleted following treatment, whereas certain taxa of the order Erysipelotrichales and phylum Actinobacteria were enriched. Cladograms were generated using Metacoder (92).
served following antituberculosis treatment were those affecting *Erysipelotrichaceae*, species of which have been associated with immune function (84) and metabolism (85). In mice, where the effect of monotherapy was analyzed, it was shown that RIF is the major driver of taxonomic alterations (57). Interestingly, in the same experiments, the mycobacterium-specific antibiotics INH and PZA, as well as EMB (S. Namasivayam, unpublished data), also independently affected the microbiota, and these changes were distinct from those observed during combination therapy. Since in wealthier countries isoniazid prophylaxis (IPT) is used frequently in adults with LTBI to prevent conversion to active disease, the effects of INH administration observed in the mouse model suggest that this group of antibiotic-treated individuals may also exhibit an intestinal dysbiosis, a hypothesis that has yet to be formally investigated.

These initial studies of the short-term effects of TB treatment on the microbiota suggest that delineation of how future TB drugs in the development pipeline affect the diversity, taxonomic structure, and metabolism of the microbiota may yield important information relevant to understanding their efficacy and potentially in predicting variations in treatment outcome between individuals.

**Chronic effects of *M. tuberculosis* treatment.** An important aspect of the TB antibiotic-induced dysbiosis is its long-lasting nature. In mice treated with HRZ, this dysbiosis lasted at least 3 months after cessation of therapy (57). In humans, active TB subjects who completed the 6-month standard course of HRZE treatment and were clinically cured for an average of at least 1.2 years displayed altered intestinal microbiome composition compared to healthy LTBI controls (81). Biomarkers of dysbiosis in treated mice were *Barnesiella*, *Porphyromonas*, *Paraprevotella*, *Parasutterella*, *Desulfovibrio*, and interestingly some *Actinobacteria*, the phylum to which mycobacteria belong. In humans, *Bacteroides*, *Faecalibacterium*, *Eubacterium*, and *Ruminococcus* were all biomarkers of dysbiosis when measured more than 1 year posttreatment.

While TB antibiotic treatment has well-defined long-term effects on the microbiome, far less is known about the possible consequences of these alterations on human health. Interestingly, after an individual is cured by TB antibiotics, their risk for reinfection is increased (86, 87) up to 4-fold in one study, suggesting a possible link between the posttreatment effects of chemotherapy on the microbiome and TB recurrence. A recent study employed an IFN-γ enzyme-linked immunospot assay to compare immune responses in patients who were treated for TB with those who were LTBI/IGRA+ but had never had active disease or received TB treatment (88). The authors found that a defined subset of *M. tuberculosis* T cell epitopes was recognized poorly by PBMCs from patients who were treated less than 6 years previously compared with PBMCs from untreated LTBI individuals. Interestingly, many of the *M. tuberculosis* epitopes in this subset had sequence homology with bacterial peptides from the Human Microbiome Project data set. In contrast, a second pool of *M. tuberculosis* epitopes with relatively weaker homology to microbiome peptides stimulated indistinguishable T cell responses in the treated TB and untreated LTBI groups. Based on these observations, the authors suggested that the cross-reactivity between certain microbiota and *M. tuberculosis* epitopes is important in maintaining long-term host resistance to TB and that the effects of TB antibiotics on these commensal taxa result in increased susceptibility to reinfection and disease. These interesting observations suggest a mechanism by which the dysbiosis induced by TB antibiotic therapy could be a risk factor for *M. tuberculosis* reinfection in cured individuals. Future analyses that incorporate matched immune readouts and microbiome measurements from the same patient to allow for direct correlations are required to validate this hypothesis.

The intestinal dysbiosis observed both during and after antituberculosis therapy may have additional consequences. For example, it may affect the absorption or metabolism of the antitycobacterial drugs themselves during the prolonged treatment regimen. Such effects could hinder or perhaps even promote the efficacy of the individual antibiotics against TB infection. Although as yet to be documented in patients, TB antibiotic treatment through the dysbiosis it induces may influence the
host resistance to other diseases. Studies employing the murine TB chemotherapy model can be used to specifically address these questions.

CONCLUSIONS

Our understanding of the interrelationship of the microbiome and TB infection and treatment is still at an early stage, and how microbiome composition and function influence *M. tuberculosis* infection and TB disease risk is far from being precisely delineated. It is becoming clear, however, that active *M. tuberculosis* infection in both mice and humans causes alterations in the microbiota, although these changes are variable between studies and in many cases of minor magnitude. In contrast, *M. tuberculosis* antibiotic treatment induces both profound and long-lasting changes in the intestinal microbiome that are largely shared between mice and humans. In mice, these changes occur within 2 weeks of treatment, the time in which *M. tuberculosis* is being rapidly cleared from the sputum in infected humans. Given these findings, critical questions that should be addressed in future studies include:

1. Does the early alteration in microbiome composition that occurs during TB treatment, which is both substantial and variable between individuals, correlate with the efficacy of TB treatment? This could be approached through prospective studies in which microbiome composition, TB bacterial load, and immune function are measured in the same subjects.

2. Are there differences in the microbiome, and immune correlates of those differences, that identify individuals resistant to initial infection or who will control latent infection?

3. Does the posttreatment dysbiosis that occurs following cessation of TB therapy have consequences for susceptibility to reinfection by *M. tuberculosis* or other pathogens, and if so, what mechanism(s) underlie this effect?

Another critical need is for improved techniques and experimental models to delineate the cross talk between *M. tuberculosis* and the lung microbiome. As discussed above, major problems exist in both the sampling of the lung flora free of oral contamination and the accurate classification of taxa due to the lower bacterial biomass in this tissue site. Interestingly, while the effects of TB antibiotics on the intestinal flora are now well documented, to the best of our knowledge, there is no information on how these antibiotics affect the lung microbiota either directly or indirectly through their effect on the removal of *M. tuberculosis* from that niche. Although TB is primarily a pulmonary disease, it is important to note that the intestinal microbiota may be equally as important as the lung microbiome in influencing pulmonary TB given its abundant size and well-known effects on systemic immunity. Indeed, studies using the mouse influenza model have shown that a homeostatic gut microbiome is critical for mounting an optimal immune response to respiratory tract flu (89) and that influenza infection can in turn affect the intestinal microbiota in a type I interferon-dependent manner (90). That a similar gut-lung axis (Fig. 1) occurs in *M. tuberculosis* infection was suggested in one report (39); however, this, as well as the above studies all employ antibiotics which in addition to affecting the intestinal microbiota could potentially alter the pulmonary flora (91). Therefore, future experimental as well as prospective clinical studies aimed at deciphering the role of the microbiome in the different stages of the TB life cycle, whenever possible, should attempt to sample both anatomical sites. Finally, given the well-documented association of host nutritional and metabolic status in TB, it is not unreasonable to predict that the microbiome, which strongly influences these physiological parameters, will also be shown to be an important factor in determining the outcome of *M. tuberculosis* infection, progression to TB disease, and risk of reinfection.

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