ABSTRACT  Members of the Mycobacterium tuberculosis complex (MTBC) have evolved causing tuberculosis (TB) in different mammalian hosts. MTBC ecotypes have adapted to diverse animal species, with M. bovis being the most common cause of TB in livestock. Cattle-to-human transmission of M. bovis through ingestion of raw milk was common before introduction of the pasteurization process. TB in humans is mainly caused by M. tuberculosis. This bacterium is considered a genetically clonal pathogen that has coevolved with humans due to its ability to manipulate and subvert the immune response. TB is a major public health problem due to airborne person-to-person transmission of M. tuberculosis. The essential yet unanswered question on the natural history of TB is when M. tuberculosis decides to establish latent infection in the host (resembling the lysogenic cycle of lambda phage) or to cause pulmonary disease (comparable to the lytic cycle of lambda phage). In this latter case, M. tuberculosis kills the host with the aim of achieving transmission to new hosts. Combating the TB epidemic requires stopping transmission. M. bovis BCG, the present vaccine against TB, is derived from M. bovis and only protects against disseminated forms of TB. Thus, a priority in TB research is development of new effective vaccines to prevent pulmonary disease. Attenuated vaccines based on M. tuberculosis as MTBVAC are potential candidates that could contribute to break the TB transmission cycle.

INTRODUCTION

Tuberculosis (TB) is the biggest killer of humanity. TB has killed more human beings than any other infectious disease in history, with an estimated loss of over a billion lives in the past 200 years (1). Despite effective treatment, in the WHO 2016 there were an estimated 10.4 million new TB cases and 1.8 million deaths attributed to the disease worldwide, surpassing those caused by AIDS (2). Still more worrying is the rising transmission of multidrug-resistant TB (MDR-TB), caused by mycobacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB drugs, isoniazid and rifampin (2). Nearly half a million new MDR-TB cases are estimated every year, which together with increasing globalization makes TB an alarming global health problem (2). Loss of compliance with the current treatments for TB raises the frightening idea of a return to the pre-antibiotic era, when 50% of TB patients died in the absence of an effective treatment. Dissemination of multi- and extremely drug-resistant Mycobacterium tuberculosis strains has adverse implications for TB control in the 21st century.

M. tuberculosis complex (MTBC) comprises a group of closely related TB-causing subspecies or ecotypes adapted to different animal hosts, including humans.
According to their phylogenetic distances, the MTBC members can be classified into eight major lineages, which include the human-adapted lineages of *M. tuberculosis* and the animal-adapted ecotypes including *M. bovis, M. caprae*, and *M. pinnipedii*, among others (3, 4). *M. tuberculosis* and humans seem to have co-evolved well before the human migrations out of Africa (5). Transmission from animals to humans has been documented throughout the history of human beings, and there is evidence that human infection caused by members of the MTBC in pre-Columbian mummies could be due to zoonotic transfer of MTBC strains by seals and sea lions between A.D. 700 and 1000 (6). This finding reconciles the presence of TB-causing bacteria in the New World prior to human colonization (6).

At the beginning of the 20th century, before the introduction of pasteurization, *M. bovis* was an important cause of TB in humans and was efficiently transmitted by ingestion of raw milk (Fig. 1). In our day, *M. bovis* is rarely found causing TB outbreaks in humans. However, some particular *M. bovis* strains have adapted to the human host with the ability to transmit between immune-compromised individuals, as discussed later in this chapter. Even in countries with a very high incidence of livestock TB caused by *M. bovis* and high incidence rates of extrapulmonary TB in humans, human infection with *M. bovis* is excluded, as different lineages of *M. tuberculosis* are considered responsible for extrapulmonary TB (7).

Nowadays, *M. tuberculosis* spread in humans is due to airborne person-to-person transmission, posing an enormous public health problem (Fig. 2). Since humans are the only known reservoir for *M. tuberculosis*, to stop the global TB epidemic, transmission must be stopped to prevent new infections and new cases (8). The traditional way to fight TB transmission is active detection of TB cases, with patients then separated safely and treated effectively. Prompt initiation of effective antibiotic treatment to rapidly render TB patients noninfectious is crucial for this task. Since TB mainly affects developing and underdeveloped countries, active case-finding is not often implemented in these regions of the world, resulting in long delays in diagnosis and treatment (9). In this context, new TB vaccines with the potential to protect against pulmonary forms of the disease could play an essential role in preventing TB transmission (Fig. 3).

**FIGURE 1.** *M. bovis* transmission. Infected cattle transmit *M. bovis* bacteria (orange bacilli) to the neighboring herd members and also during milking. Before milk pasteurization was introduced, *M. bovis* was an important cause of cattle-to-human transmission of TB. Now *M. bovis* rarely causes TB outbreaks in humans, and transmission of *M. bovis* strains between humans is infrequent. Vaccination with BCG (schematized as blue bacilli), starting in the 1920s, was efficient to prevent disseminated forms of TB caused by *M. bovis*. 

**FIGURE 2.** Cattle-to-cattle transmission of *M. bovis*. 

**FIGURE 3.** Prevention of disseminated forms of TB caused by *M. bovis*.
TB INFECTION OR TB DISEASE: THE M. TUBERCULOSIS DECISION TO LIVE WITH OR TO KILL ITS HOST

In humans, the M. tuberculosis infectious cycle starts with the transmission of the tubercle bacilli by the respiratory route from a patient with active pulmonary disease, who aerosolizes M. tuberculosis, placing contacts at risk of infection. Epidemiological data indicate that 9 of every 10 infected individuals are chronically infected in the form of LTBI (gray human shapes); therefore, LTBI constitutes a potential reservoir for transmission. People with LTBI are at risk for TB reactivation at some later time, and 1 of every 10 infected persons will develop clinical disease (black human shapes). The essential question on the natural history of TB is when M. tuberculosis decides to either infect and live with its host in the form of LTBI or to cause active pulmonary disease, which without treatment kills the host, searching the transmission to new hosts. The inner circle shows the lambda phage infectious cycles and their similarities to M. tuberculosis infection and disease. The lysogenic cycle of lambda phage resembles to LTBI, and the lytic cycle of lambda phage is similar to active TB disease caused by M. tuberculosis.

FIGURE 2 M. tuberculosis transmission. Humans are the only known reservoir of M. tuberculosis (red bacilli). The M. tuberculosis infectious cycle starts with the transmission of bacilli by the respiratory route from a patient with active pulmonary disease, who aerosolizes M. tuberculosis, placing contacts at risk of infection. Epidemiological data indicate that 9 of every 10 infected individuals are chronically infected in the form of LTBI (gray human shapes); therefore, LTBI constitutes a potential reservoir for transmission. People with LTBI are at risk for TB reactivation at some later time, and 1 of every 10 infected persons will develop clinical disease (black human shapes). The essential question on the natural history of TB is when M. tuberculosis decides to either infect and live with its host in the form of LTBI or to cause active pulmonary disease, which without treatment kills the host, searching the transmission to new hosts. The inner circle shows the lambda phage infectious cycles and their similarities to M. tuberculosis infection and disease. The lysogenic cycle of lambda phage resembles to LTBI, and the lytic cycle of lambda phage is similar to active TB disease caused by M. tuberculosis.

In humans, the M. tuberculosis infectious cycle starts with the transmission of the tubercle bacilli by the respiratory route from a patient with active pulmonary disease, placing their contacts at risk of infection (10). Efficient transmission of TB is dependent on the generation of a lesion in the lung, which results in a bacterium-laden cough (Fig. 2). When a patient with pulmonary TB coughs, sneezes, or even speaks, the aerosolized M. tuberculosis can be inhaled by neighboring individuals; bacteria reach the alveoli, where resident macrophages phagocytose them. M. tuberculosis has adapted to an intracellular lifestyle; the fate of most bacteria in the phagosome is often death, but M. tuberculosis has developed adaptations to survive and even to escape from the macrophage phagosome. M. tuberculosis is able to manipulate both the innate and acquired immune responses of the host. This manipulation results in an effective CD4+ T-cell response that limits bacterial dissemination but can also promote the development of a progressive and destructive lesion in the lungs (11).
The development of lesions in pulmonary disease, the most common form of TB, is the result of the conflict between the invader, *M. tuberculosis*, and the host. TB is the paradigm for diseases caused by an intracellular pathogen. The pathogen is able to multiply within macrophages and monocytes, and the host immune system is thought to control the infection by cell-mediated immunity (CMI) carried out by effector-activated macrophages orchestrated by T-cell-derived lymphokines. The CMI generated in TB is so potent that on average 90% of immunocompetent humans infected with *M. tuberculosis* are able to contain the infection as latent TB infection (LTBI) (Fig. 2) and avoid progression to clinical disease during their lifetimes (12). It is estimated that >2 billion humans have LTBI, and most of them remain infected although asymptomatic. People with latent TB are at risk of disease reactivation at some later time due to a weakened immune system (e.g., as a result of malnutrition, stress, diabetes, or HIV infection), making LTBI individuals potential reservoirs of transmission (Fig. 2). The risk of progression to active TB is higher in the several years following initial infection. The extent to which LTBI reduces the risk of progression to active TB following reexposure and reinfection has been recently reviewed by Andrews et al. (13), who concluded that individuals with LTBI had a 79% lower risk of progressive TB after reinfection than uninfected individuals. Taken together, pulmonary cases of TB represent the tip of the iceberg when compared with the one-third of the world population latently infected with TB.

In a minority of cases (5 to 10% of infected individuals), the caseum of the TB lesion softens and infection with *M. tuberculosis* progresses into TB disease. The intracellular bacilli avoid killing by macrophages by arresting phagosome-lysosome fusion. *M. tuberculosis* is also able to escape from the phagosome to the cytosol, where it continues multiplying, eventually leading to lysis of the infected cell. These extracellular bacilli are then taken up by other macrophages and by blood monocytes that are attracted to the infectious focus and then develop into immature macrophages. The latter cells readily ingest bacilli but are incapable of killing virulent *M. tuberculosis* organisms or inhibiting their growth. This results in the accumulation of an extracellular bacillary population growing in the lung cavity. The tuberculous lung cavity with its high bacillary content is discharged into the bronchi and subsequently to other parts of the lung and to the outside environment, allowing human-to-human transmission. Before the antibiotic era, 50% of patients with cavitary lung TB died within 2 years (12). Efficient coevolution of *M. tuberculosis* with its human host has led to a fine-tuned balance between latency, a persistent phase of the infection responsible for a massive reservoir from which active...
TB cases may emerge, and the infamous ability of the bacterium to cause severe lung pathology that is a prerequisite for aerosol spread (14).

*M. tuberculosis* has the ability to promote dynamic responses to the host environment in order to guarantee its survival, replication, and transmission. Determinants of *M. tuberculosis* virulence include biologically active secreted proteins such as ESAT-6 (early secretory antigenic target-6), which mediates damage to the mycobacterial phagosomal membrane, which appears to enable the bacteria to replicate in the cytosol, allowing cell-to-cell spread of virulent *M. tuberculosis* (15, 16). Active lipids interact with the host to contribute to the long-term success of the bacteria by modulating intracellular bacterial trafficking, host cell death pathways, and granuloma formation (17).

Some risk factors leading to progression to active disease in LTBI individuals are related to malnutrition and immunodeficiency of the host. Coinfection with other pathogens such as HIV unbalances the CMI of the host and profoundly alters the immune response to *M. tuberculosis* (18). Treatment for diseases such as rheumatoid arthritis and Crohn’s disease with TNF antagonists have been found to markedly increase the frequency of TB reactivation. Further, there is increasing evidence highlighting the importance of vitamin D as a potent modulator of human immune responses, with important implications for the control of TB infection (19). When does *M. tuberculosis* take the decision to remain as LTBI or to progress to disease, allowing TB transmission? And how is this decision taken? Both are essential unresolved questions about the natural history of TB, and their answers will greatly help to discover methods to allow control of respiratory transmission of *M. tuberculosis*.

Members of the MTBC are a highly clonal population, which is particularly well reflected by the evolutionary hyperconservation of human T-cell epitopes. Most bacterial pathogens rely on antigenic drift to favor immune escape. However, the hyperconservation of T-cell epitopes in *M. tuberculosis* implies a better recognition of the pathogen by the human immune system, allowing it to subsequently decide whether to cause disease or remain latent (19). Probably this could reflect the need for *M. tuberculosis* to develop pulmonary forms of the disease where bacteria are able to grow extracellularly, allowing transmission to new hosts.

It is tempting to think that *M. tuberculosis* is acting with its human host in the same way that bacteriophages act with their bacterial hosts (Fig. 2). Lambda phage integrates its genome in the *Escherichia coli* chromosome during the lysogenic cycle, ensuring transmission of the prophage every time when bacteria divide. This lysogenic phase reminds us of the LTBI with *M. tuberculosis* dialogue with the host conferring advantages as an strong CMI against other infections. When the phage senses that its bacterial host is in danger as a result of starvation or DNA damage, e.g., by UV light among others, lambda decides to start the lytic cycle, producing multiple copies of its genome and the synthesis of its own proteins, causing the destruction of the bacterial host and transmitting to other bacteria. This lytic phase is similar to the active phase of TB disease, where mycobacteria multiply extracellularly, allowing the transmission to a new host (Fig. 2). Similar to lambda phage that senses a damaging environment of its host, *M. tuberculosis* produces active disease when the immune status of the host is impaired, as in the case of an HIV infection when CMI declines, diminishing the CD4 T-cell count. Today, more than 6 decades after the initial discovery of the lambda phage, we have come to decipher its molecular life cycle with *E. coli*. Hopefully, in the very near future, new technologies and smart researchers will allow us to decipher the intimate relationship of *M. tuberculosis* with the human host and to identify the warning stimuli that awaken latent *M. tuberculosis* to complete its transmission cycle.

**TRANSMISSION OF MTBC LINEAGES AND ADAPTATION TO THE HOST LIFESTYLE**

Comparative genome analyses have identified a series of regions of difference (RDs), also named large sequence polymorphisms (LSPs), which due to their irreversible nature and intralineage conservation have allowed proposal of broad and accurate evolutionary schemes of the *M. tuberculosis* complex that are still valid (3, 20, 21). These studies suggest a strong indication for a clonal population structure of the MTBC, without evidence of ongoing horizontal gene transfer (3). This has made possible the development of molecular methods to characterize circulating strains, such as gold standard restriction fragment length polymorphism (RFLP) analysis (22, 23), spoligotyping (24), mycobacterial interspersed repetitive-unit-variable-number tandem-repeat analysis (25), and/or multilocus sequencing approaches (26). These molecular epidemiology techniques are valuable tools to study TB transmission patterns suspected from traditional epidemiological investigation, allowing characterization of highly transmissible *M. tuberculosis* strains. The emergence of whole-genome sequencing more recently as an affordable tool for studying genomic
content and inferring genetic relationships offers an extraordinarily detailed view on the evolution of M. tuberculosis lineages and outbreak clones, with unprecedented possibilities for verification, interpretation, and refinement of hypotheses on the distant and recent evolution of the tubercle bacilli (14).

Comparative genomics has resulted in congruent groupings of MTBC comprising eight major lineages, which include the human-adapted ecotypes M. tuberculosis (L1 to L4 and L7), M. africanum (L5 and L6), and M. canettii, and the animal-adapted ecotypes grouped into L8 (4). According to RD distribution and whole-genome data, the animal-adapted lineages of the MTBC seem to have evolved from RD9-deleted M. africanum-like ancestor strains that may well have been adapted to humans. As regards the presence of L2, L3, and L4 lineages, these TbD1-deleted lineages might correspond to “modern” M. tuberculosis strains that were introduced into Africa by more recent human contact and colonialism (14).

Epidemiological data suggest that the different phylogenetic lineages of MTBC might have adapted to different human populations. Over all, the phylogenetically “modern” MTBC lineages are more successful in terms of their geographical spread compared with the “ancient” lineages. Interestingly, the global success of modern MTBC correlates with a hypoinflammatory phenotype in macrophages, possibly reflecting higher virulence, and a shorter latency in humans. Finally, various human genetic variants have been associated with different MTBC lineages, suggesting an interaction between human genetic diversity and MTBC variation (8). M. tuberculosis organisms belonging to lineages 2, 3, and 4 are more closely related and well extended in urban populations. Contacts exposed to modern MTBC are more likely to develop active TB in a shorter time compared with individuals exposed to ancient MTBC, who develop TB later (27). Additionally, it has been recently found that genetically diverse strains of MTBC vary widely in induction of an early inflammatory response during infection of human macrophages, with a significantly lower response to evolutionarily modern lineages as compared with ancient lineages (19, 28). It would seem that virulence of the strains may be related to populations living in either rural areas, where transmission needs to be slow to ensure dissemination to the next generation, or crowded cities, where population densities are high enough to allow fast transmission of the disease between neighboring individuals.

Host-pathogen coevolution is characterized by reciprocal adaptive changes in interacting species. Host immune pressure and associated pathogen immune evasion are key features of this process, often referred to as an evolutionary arms race. Studies in human pathogenic viruses, bacteria, and protozoa have revealed that genes encoding antigens tend to be highly variable as a consequence of diversifying selection to evade host immunity. However, since all MTBC strains have highly conserved human T-cell epitopes, the ultimate transmission mechanisms rely on the host immune response that contributes to tissue destruction and formation of cavities in the lung (8).

MDR-TB OUTBREAKS: THE RARE CASE OF A PARTICULAR M. BOVIS MDR-XDR STRAIN TRANSMITTED BETWEEN HUMANS

Since no plasmids or horizontal gene transfer for antibiotic resistance genes have been detected, M. tuberculosis acquires antibiotic resistance by selection of specific mutations in target genes (3). Transmission of MDR-TB (resistant at least to isoniazid and rifampin) strains depends on the fitness cost of the mutations conferring the drug resistance phenotype. In laboratory-derived mutants of M. tuberculosis, rifampin resistance could be associated with a competitive fitness cost, and this cost is likely determined by the specific resistance mutation and strain genetic background. In the case of epidemic MDR M. tuberculosis clinical isolates, no fitness-impairing mutations have been found (29). Isoniazid-resistant isolates have been used to demonstrate that strain genetic diversity influences the transmission dynamics of drug-resistant bacteria (30). Association between specific drug resistance mutations and the main M. tuberculosis lineages allows us to predict that strain fitness is an important determinant of MDR M. tuberculosis transmission and demonstrates the important effects of strain diversity. The impact of resistance mutations on the transmission of isoniazid-resistant M. tuberculosis is very important; for example, strains with a KatG S315T or inhA promoter mutation were more likely to spread than strains with other mutations that have lost their fitness.

In the 1990s, an MDR-TB outbreak was extended from >200 New York City patients and to at least four additional U.S. cities, and transmitted to Europe. The MDR M. tuberculosis clonal strain, named “W strain,” belongs to lineage 2 (Beijing family). Isolates were typed by IS6110-based RFLP, and gene mutations associated with resistance to rifampin and isoniazid were studied. The MDR phenotype in these organisms arose by sequential acquisition of resistance-conferring mutations in several genes, most likely as a consequence of anti-
biotic selection of randomly occurring mutants in concert with inadequately treated infections (31). The MDR-TB epidemic was the confluence of several factors, including the AIDS epidemic as result of HIV infection, before an effective antiretroviral treatment was available; immigration from countries where TB is endemic; poverty; homelessness; and lack of TB control programs.

In Europe, a high rate of TB reinfection during a nosocomial outbreak of MDR-TB caused by M. bovis was detected in the 1990s among AIDS patients (32). The M. bovis strain was typed by IS6110-based RFLP and named “strain B,” and after studying the resistance phenotype of the strain, it was classified as extensively drug-resistant TB (XDR-TB), which is an MDR-TB further resistant to any fluoroquinolone and to at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (33). Strain B caused >100 deaths of AIDS patients in different regions throughout Spain (33). Since M. bovis exhibits a lower transmissibility among humans than M. tuberculosis sensu stricto (Fig. 1), it seems very likely that the higher human-to-human transmission rate of strain B was a consequence of additional fitness gained by this particular isolate. Molecular epidemiology techniques identified the insertion of an IS6110 mobile element upstream of phoPR, leading to overexpression of this operon, which codes for the two-component system PhoPR, which is essential for the virulence of M. tuberculosis (34). This finding suggested that PhoPR-associated phenotypes likely favored aerosol transmission of M. bovis strain B between humans (35). The discovery of the essential role of the phoP gene in this outbreak of MDR-TB was the founding principle for the construction of the attenuated vaccine MTBVAC, now being evaluated in the clinic, as discussed in detail below.

ANIMAL MODELS TO STUDY TB TRANSMISSION

In view of the great difficulty of monitoring TB transmission among humans, future research efforts might be well advised to focus on animal models of transmission (36). The relationship between TB transmission dynamics and the evolution of M. tuberculosis has been an important question in TB research for many years. This has not been studied in TB because of the lack of an experimental TB transmission model in a well-controlled system.

One important line of research in TB is the relationship between transmissibility and bacterial genotype. A transmission mouse model has been established to study the hypothesis that increased virulence of the strains was linked to increased transmission (37). By using a BALB/c mouse model of progressive pulmonary TB, authors examined the course of infection in terms of strain virulence (mouse survival, lung bacillary load, and histopathology) and immune response cytokine expression produced by different M. tuberculosis strains selected from clinical/epidemiological studies. Animals that were exposed to the more virulent strain developed the hallmark of progressive disease (pneumonic patches) after 2 months of cohousing, whereas the low- or intermediate-virulence strains only induced small granulomas and chronic inflammatory infiltrates. A correlation between virulence, immunopathology, and transmissibility of selected strains of M. tuberculosis was found. The research was able to correlate virulent and transmissible phenotypes and markers of community transmission such as tuberculin reactivity among contacts, rapid progression to disease, and cluster status. Nevertheless, as mice lack the cough reflex, this animal model is not considered to mirror the TB transmission mechanisms occurring in humans.

In the case of guinea pigs, due to their high susceptibility to TB, their use as sentinels in hospitals to study direct transmission from human TB patients has been explored (38).

Quantitative studies of disease in animals that develop cavitary, transmissible TB may be key in determining whether the host or the pathogen plays the role in TB transmission. In this regard, an interesting model in cattle has been established to study transmission of bovine TB in a natural transmission setting to examine the efficacy of M. bovis BCG for protection against bovine TB in calves under field conditions. The study demonstrated a BCG protective efficacy between 56 and 68% (39).

Goats are highly social and relatively easy-to-handle animals and have been proposed as a reliable ruminant model for research, representing an easier approach than use of larger ruminants (40). Goats are very sensitive to TB infection, exhibiting disease-causing lesions in the lungs similar to humans (41) when infected with different members of the MTBC, including M. bovis and M. caprae (42). Recent studies have demonstrated an effective and quick rate of TB transmission in this animal model by cohousing TB-infected and -noninfected goats (43). Today, goats represent a very promising ruminant model for the assessment of vaccine efficacy against TB transmission (L. Dominguez, personal communication).
BCG: THE ONLY VACCINE IN USE TO PREVENT DISSEMINATED BUT NOT RESPIRATORY FORMS OF TB

BCG is a live attenuated vaccine derived from *M. bovis*, isolated for the first time in 1902 by Edmond Nocard from the milk of a cow suffering from tuberculous mastitis. BCG is almost 100 years old and is currently the only available vaccine for the prevention of human forms of TB. BCG is effective for the disseminated forms of TB and is included as part of the routine immunization schedule in developing countries. Since 1974, BCG vaccination at birth has been included in the World Health Organization Expanded Programme on Immunization, resulting in more than 3 billion cumulative vaccinations worldwide. Today, BCG is the most widely administered vaccine in humans, being one of the vaccines with minimal recorded adverse events.

Since BCG protects against the more severe forms of TB (meningitis and miliary TB) in children, thousands of lives are saved every year (44). The coverage of BCG is nearly 90% all around the world, and >100 million doses are administered every year (44, 45). However, BCG is very variable in protecting against pulmonary TB, responsible for TB transmission. Consequently, BCG is not recommended for use in the United States or other high-income countries considered to have a low burden of TB.

Multiple explanations have been put forward to explain the variable efficacy of the BCG vaccine. It is very likely that BCG is not able to establish effective central memory T cells. Accordingly, BCG protects infants and adolescents for 10 to 15 years, but protection is gradually lost in adults. This may have extraordinary implications for developing new TB vaccines aiming to enhance central memory immunity (46).

As previously described, the experimentally verified human T-cell epitopes of *M. tuberculosis* are the most conserved elements of its genome (19). The genome sequences of different BCG strains were compared to determine T-cell epitope conservation (47). It was found that among the 1,530 human T-cell epitopes, 23% of them are absent in the BCG vaccines. The majority of the absent epitopes in BCG are contained in three proteins: ESAT-6, CFP-10 (10-kDa culture filtrate protein), and PPE68 (cell wall protein from PPE family that contributes to *M. tuberculosis* maintenance of infection) all of them encoded in the RD1 region absent in BCG (47).

If these epitopes are necessary to complete the pulmonary cycle of *M. tuberculosis*, we could hypothesize that vaccine candidates containing epitopes absent in BCG could confer immunity to protect against pulmonary TB.

THE SEARCH FOR A NEW TB VACCINE TO END TRANSMISSION

In the last 15 years, many vaccine candidates have entered clinical trials. Most are protein-adjuvant formulations and recombinant viral-vectored constructs designed to increase the protection from pulmonary TB in individuals previously vaccinated with BCG (prime-boost strategy) (18). Two live attenuated whole-cell vaccines are in clinical trials for prime vaccination, one based on recombinant BCG and the other based on attenuated *M. tuberculosis* of human origin (18).

Live, rationally attenuated MTBVAC is a derivative of an *M. tuberculosis* isolate belonging to lineage 4 (Euro-American), one of the most widespread lineages of *M. tuberculosis* today. MTBVAC contains antigens present in *M. tuberculosis* strains commonly transmitted between humans by the aerosol route, including those antigens deleted in the RD1 region of *M. bovis* BCG (ESAT-6, CFP-10, and PPE68). MTBVAC contains two independent stable deletion mutations in the virulence genes *pboP* and *fadD26*. These deletions were generated in the absence of antibiotic resistance markers, fulfilling the Geneva consensus requirements for progressing live mycobacterial vaccines to clinical trials (48). PboP is a transcription factor that controls expression of 2% of the *M. tuberculosis* genome, including production of immunomodulatory cell wall lipids and ESAT-6 secretion (4). Deletion of *fadD26* leads to complete abrogation of synthesis of the virulence surface lipids phthiocerol dimycocerosates. Extensive preclinical studies with the prototype vaccine S02 in mice, guinea pigs, and nonhuman primates (49–51) and final vaccine candidate MTBVAC in mice and guinea pigs have demonstrated attenuation and safety of MTBVAC comparable to BCG, with superior immunogenicity and efficacy against *M. tuberculosis* (48, 52). A first-in-human MTBVAC clinical trial was recently completed successfully in healthy adults in Lausanne, Switzerland (NCT02013245) (53). In this trial, when MTBVAC was given at the same dose as BCG (5 × 10⁵ CFU), there were more responders in the MTBVAC group than in the BCG group, with a greater frequency of polyfunctional CD4⁺ central memory T cells. However, this study has the limitation, as a phase 1 first-in-human trial, that the secondary objective (immunogenicity) was not powered for statistical analysis. Nevertheless, MTBVAC is the first live attenuated *M. tuberculosis* vaccine to enter clinical trials and to date has shown a comparable safety profile to BCG. A notable finding in the first trial was a transitory ESAT-6- and CFP-10-specific T-cell responses that at the end of the study was negative, sug-
gesting that gamma interferon release assays could be utilized as study endpoints in future efficacy trials to test efficacy against M. tuberculosis infection (53). The immunogenicity data show that MTBVAC is at least as immunogenic as BCG, a result congruent with the maintenance of the whole epitope repertoire from an M. tuberculosis strain. Altogether, these data supported the advanced clinical development in high-burden countries where TB is endemic. A dose-escalation safety and immunogenicity study to compare MTBVAC to BCG in newborns with a safety arm in adults is currently ongoing in South Africa (NCT02729571). A review on MTBVAC, from discovery to clinical trials in tuberculosis-endemic countries, has been recently published (54).

Due to the lack of a correlation of protection for TB, future efficacy studies with MTBVAC in countries with a high incidence of TB could demonstrate the effectiveness of this vaccine in preventing pulmonary forms of TB. Efficacy studies in new TB transmission animal models, such as the recently developed model in goats (55), are extremely important to demonstrate proof of concept of pulmonary protection to help accelerate clinical development of new TB vaccines toward efficacy trials in humans. Specific response in human to TB vaccinees candidates as potential biomarker of protection could accelerate the clinical trials efficacy evaluation (56).

CONCLUDING REMARKS

Additional threats to TB control include the spread of MDR-TB, the appearance of XDR-TB, and the destructive impact of TB/HIV coinfection. Today, the main strategy to stop TB transmission is via active case-finding and by prompt establishment of an effective treatment as soon as TB is diagnosed. New vaccines against M. tuberculosis are essential for preventing infection, disease, and transmission. The development of a new vaccine able to protect against pulmonary forms of TB is essential to overcome this terrible disease, with the final objective to eradicate TB from the planet.

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CONFLICT OF INTEREST

C.M., J.G.-A., and N.A. are coinventors of a patent on a live attenuated TB vaccine held by the University of Zaragoza. There is not any commercial or other association that might pose a conflict of interest.

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