

Review

Mycobacterium tuberculosis: ecology and evolution of a human bacteriumAnne-Laure Bañuls,^{1,2} Adama Sanou,¹ Nguyen Thi Van Anh² and Sylvain Godreuil^{3,4,5}

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Some species of the *Mycobacterium tuberculosis* complex (MTBC), particularly *Mycobacterium tuberculosis*, which causes human tuberculosis (TB), are the first cause of death linked to a single pathogen worldwide. In the last decades, evolutionary studies have much improved our knowledge on MTBC history and have highlighted its long co-evolution with humans. Its ability to remain latent in humans, the extraordinary proportion of asymptomatic carriers (one-third of the entire human population), the deadly epidemics and the observed increasing level of resistance to antibiotics are proof of its evolutionary success. Many MTBC molecular signatures show not only that these bacteria are a model of adaptation to humans but also that they have influenced human evolution. Owing to the unbalance between the number of asymptomatic carriers and the number of patients with active TB, some authors suggest that infection by MTBC could have a protective role against active TB disease and also against other pathologies. However, it would be inappropriate to consider these infectious pathogens as commensals or symbionts, given the level of morbidity and mortality caused by TB.

Introduction

Tuberculosis (TB) is a contagious infectious disease caused in humans mainly by *Mycobacterium tuberculosis* (MTB). MTB is spread essentially through the air: when an infectious person coughs, sneezes, talks or spits, saliva droplets containing tubercle bacilli are projected into the air and can be inhaled by a nearby person. Indeed, tubercle bacilli enter the human body mainly through the respiratory route after inhalation of these tiny droplets expelled into the air (Fig. 1). These particles are small enough to be able to reach the lower airways (Dannenberg, 1991). The infection success and the development of the pulmonary form of TB (lungs are the main target of this bacterium) depend on four successive steps: phagocytosis of the bacilli, their intracellular multiplication, the latent contained phase of infection and finally the active lung infection. These steps can progress towards different clinical

scenarios: spontaneous cure, disease, latent infection and re-activation, or re-infection (see Fig. 1; reviewed by Godreuil *et al.*, 2007b). Immunosuppressed individuals are more at risk of developing active TB once infected, particularly patients with AIDS. However, none of the critical (host- or pathogen-related) determinants involved in the clinical outcome of human immunodeficiency virus (HIV)/TB co-infection is known in detail (Godreuil *et al.*, 2007a; Pean *et al.*, 2012; Laureillard *et al.*, 2013; Marcy *et al.*, 2014).

History suggests that TB appeared about 70 000 years ago (see below) and that it remained sporadic up to the 18th century. It then became epidemic during the industrial revolution, owing to the increased population density and the unfavourable living conditions. During the 20th century, TB incidence started to decrease rapidly in developed countries thanks to improvement of health, nutrition and housing conditions. TB incidence reduction became even more rapid following the introduction of the BCG (*Bacillus Calmette–Guérin*) vaccine in 1921 and the use of antimicrobial drugs, such as streptomycin (1943), isoniazid (1952) and rifampicin (1963). However, despite

Abbreviations: AIDS, acquired immunodeficiency syndrome; MTB, *Mycobacterium tuberculosis*; MTBC, *Mycobacterium tuberculosis* complex; TB, tuberculosis; WHO, World Health Organization.

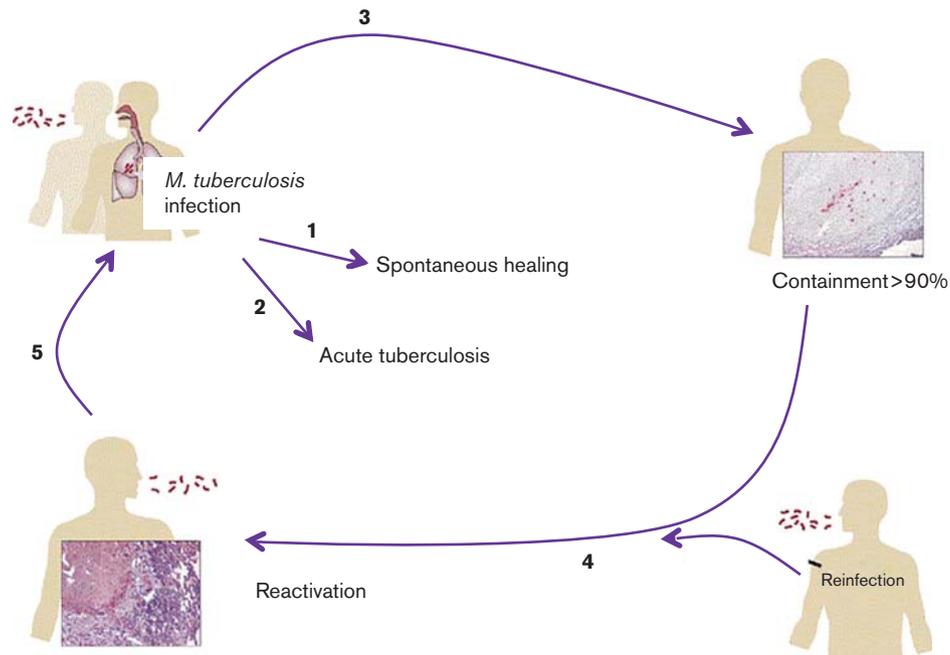


Fig. 1. Cycle based on Kaufmann *et al.* (2006) and Godreuil *et al.* (2007b). MTB enters the host by inhalation of aerosols. Different scenarios are possible: (1) immediate elimination of MTB by the pulmonary immune system; (2) infection progresses to active tuberculosis; (3) infection does not progress to active disease and MTB enters a latency phase; (4) after the latency phase, MTB can become active following endogenous reactivation or a new exogenous infection or both; (5) at this stage, there is MTB dissemination and transmission.

efforts to eradicate this disease, TB incidence increased again in the 1980s, owing to the HIV pandemic, the deterioration of health conditions in large cities and the appearance of resistance to antibiotics. During the last 100 years, TB has probably killed more than 100 million people (Frieden *et al.*, 2003). It is a major public health problem worldwide because about one-third of the world population has latent TB, representing the natural reservoir of this pathogen. Moreover, almost 9 million people have active TB and 2 million die from this disease each year. More than 90 % of TB cases occur in developing countries and the regions most concerned by this disease are Africa, South-East Asia and East Europe. Several parameters are involved in its maintenance and recrudescence, such as social and health conditions, the association with HIV/AIDS (1.2 million patients are co-infected by MTB/HIV), the reduced efficacy of the BCG vaccine, the movement of populations, and the existence of multidrug-resistant strains (worldwide, 500 000 cases of TB are due to multidrug-resistant strains and 27 000 cases to extensively drug-resistant strains). Indeed, 70 years ago, there was no drug to treat TB. Currently, the number of available antibiotics has considerably increased, but the first antibiotics discovered during the 1950s and 1960s still are first-line TB treatments, particularly rifampicin and isoniazid (WHO, 2014). The appearance of drug-resistant and multidrug-resistant TB strains (rifampicin and isoniazid

resistant, and now also of extensively and totally drug-resistant strains) makes the management of this disease very difficult. The current treatment recommended by the World Health Organization (WHO) to control the drug-resistance problem includes several phases with different combinations of antibiotics. However, the complexity and long duration (at least 6 months) (WHO, 2014) of the treatment make its application in low-income countries difficult. Moreover, drug sensitivity tests are not carried out routinely in many countries, particularly in developing countries. Bad treatment compliance (incomplete or poorly followed treatment) and lack of effective and rapid diagnostic tools are the major factors of emergence and transmission of drug-resistant TB in populations.

A more effective vaccine against pulmonary TB, which is the source of disease transmission, is crucially needed. Currently, the only available vaccine is the BCG vaccine made of an attenuated strain of *Mycobacterium bovis* that has lost its virulence (Oettinger *et al.*, 1999). In their review, Rook *et al.* (2005) discuss BCG limits, its efficacy for the severe forms of TB in young children (TB meningitis and disseminated TB), its variable protection in adults and its weak impact in developing countries. During the last 20 years, hundreds of candidate vaccines have been under study; however, none has passed the clinical trial phase (Ginsberg, 2002; Hampton, 2005; Kaufmann *et al.*, 2006; Kaufmann, 2011, 2012, 2013).

MTB and the MTBC: a history of hosts

To come to an understanding of the current situation, we must talk about MTB and the complex to which it belongs, MTBC. This complex includes several *Mycobacterium* species with nearly identical nucleotide sequences and totally identical 16S rRNA sequences. This extreme similarity proves that they all have a common ancestor. However, they differ in terms of host tropism, phenotypes and pathogenicity. Indeed, some of these species are human pathogens (MTB, *Mycobacterium africanum*, *Mycobacterium canettii*) or rodent pathogens (*Mycobacterium microti*). Others infect seals and sea lions (*Mycobacterium pinnipedi*), or sheep and goats (*Mycobacterium caprae*), while *M. bovis* has a larger spectrum of host species, including bovids and humans.

Even among the *Mycobacterium* species that are more specifically confined to humans and that we shall call human MTBC, very different genomic, phenotypic, clinical and epidemiological features can be observed. MTB and *M. africanum*, for instance, have circular genomes ranging between 4.38 and 4.42 Mb, while the *M. canettii* genome is 10–115 kb larger, representing the tubercle bacillus with the biggest genome. Phenotypically, *M. canettii* forms smooth colonies, different from all the other MTBC species, which produce rough colonies. To differentiate them, Supply *et al.* (2013) have called these bacilli ‘smooth tubercle bacilli’. MTB is present everywhere in the world, while *M. africanum* is localized specifically in Africa (de Jong *et al.*, 2010) and *M. canettii* seems to be confined to the Horn of Africa (Miltgen *et al.*, 2002; Fabre *et al.*, 2010; Koeck *et al.*, 2011). *M. africanum* and MTB are microorganisms with a specific tropism for humans and cause mainly pulmonary TB. No animal reservoir has been confirmed, although some cases of bovine TB caused by MTB have been reported (Gidel *et al.*, 1969; Rey *et al.*, 1986; A. Sanou, Z. Tarnagda, E. Kanyala, D. Zingué, M. Nouctara, H. Hien, N. Meda, P. Van de Perre, A. L. Bañuls & S. Godreuil, unpublished data). It is interesting also that recent works have demonstrated that MTB, like *M. bovis* and *M. canettii*, can survive in soil for long periods of time and maintain its infectious potential (Ghodbane *et al.*, 2014). MTB and *M. africanum* must, generally, cause active pulmonary TB to be transmitted from one host to another. *M. canettii* epidemiology is completely different. This bacterium was described for the first time in the 1960s and only about 100 strains have been isolated so far (Fabre *et al.*, 2010). Clinical cases remain rare and they mostly are extra-pulmonary forms of TB. Up to now, inter-human transmission has not been demonstrated. All this suggests the existence of an environmental reservoir (Koeck *et al.*, 2011).

Although Hershberg *et al.* (2008) have demonstrated that MTBC genetic diversity is more important than initially described, particularly for MTB, it is at an extremely low level compared with other bacterial models (Hershberg *et al.*, 2008). *Mycobacteria* present one of the most extreme examples of genetic homogeneity, with about 0.01–0.03 % synonymous nucleotide variation (Gutierrez *et al.*, 2005).

It is interesting to note that *M. canettii* shows a bigger global genetic diversity than other MTBC members, although very few strains have been isolated and its spatial distribution seems to be limited to the Horn of Africa. Moreover, although MTB propagation seems to be clonal (absence of horizontal genetic exchange), some studies have demonstrated the existence in *M. canettii* of frequent horizontal genetic transfers (Koeck *et al.*, 2011; Supply *et al.*, 2013). According to Supply *et al.* (2013), horizontal exchanges could occur also between *M. canettii* and MTB.

These features indicate that all MTBC species share a common origin. Nevertheless, their distinct biological, ecological and clinical characteristics suggest different evolutionary paths. Phylogenetic studies have brought some insights into how the MTBC developed. Gagneux & Small (2007) in their review article explain in detail how the MTBC lineages have been defined. Briefly, within MTB, five major lineages that are associated with different regions of the world have been described: lineage 1 (East Africa, Philippines, in the region of the Indian Ocean), lineage 2 (East Asia), lineage 3 (East Africa, Central Asia), lineage 4 (Europe, America, Africa) and, very recently, lineage 7 in Ethiopia (Firdessa *et al.*, 2013). *M. africanum* has been split in two phylogenetically distinct lineages: lineage 5 (West African 1) and lineage 6 (West African 2). Animal species also represent individualized monophyletic lineages. Finally, *M. canettii* has an ancestral position within the MTBC (see Fig. 2, and Brosch *et al.*, 2002; Comas *et al.*, 2013; Supply *et al.*, 2013).

The ‘ancientness’ of these lineages relative to each other has been determined based on the study of 20 variable genomic regions that are the result of insertion/deletion events (Brosch *et al.*, 2002). Particularly, on the basis of the presence or absence of an MTB-specific deletion (TbD1), these lineages could be subdivided into ancestral (TbD1⁺) or modern lineages (TbD1⁻). This subdivision has been clearly confirmed by other studies [see the review by Gagneux (2012)]. Lineages 1, 5, 6 and 7, ‘the animal’ lineages (*M. bovis*, *M. microti* and *M. pinnipedi*) and *M. canettii* cluster in the ancestral MTBC group (TbD1⁺) (see Fig. 2), whereas lineages 2, 3 and 4 constitute the modern group (TbD1⁻). Based on these works, the authors have also shown that, contrary to the established hypothesis, the animal lineages (*M. bovis* and *M. microti*) and *M. africanum* have diverged from the progenitor of the ancestral MTB lineages. The finding that *M. canettii* and the ancestral MTB lineages do not show any of the deletions observed in the animal lineages suggests that the tubercle bacillus was originally a human pathogen. This hypothesis has also been supported by more recent work based on genome-wide analyses (reviewed by Gagneux, 2012; Comas *et al.*, 2013). It is interesting to note that lineage 7 represents an intermediate phylogenetic branch between ancient and modern MTB lineages (Firdessa *et al.*, 2013).

All MTB lineages can then be subdivided in families or SITs (spoligotype international types) by spoligotyping, the

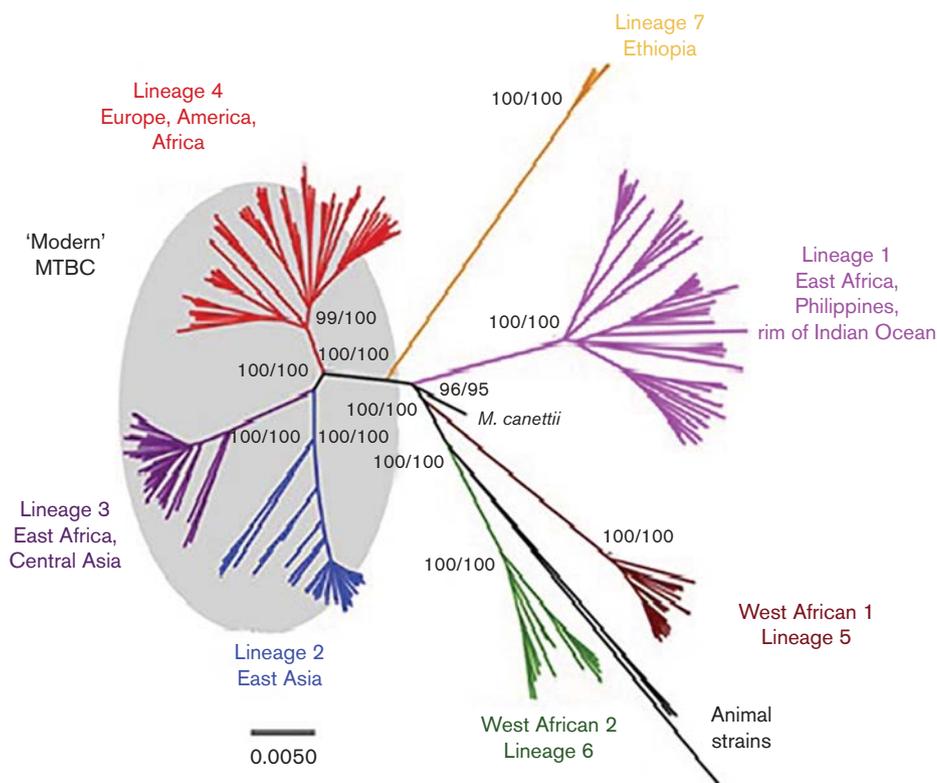


Fig. 2. Phylogeny of the MTB complex based on genome-wide studies (derived from Comas *et al.*, 2013). The five MTB lineages (lineages 1, 2, 3, 4 and 7) are represented. The figure highlights the two individualized lines of *M. africanum* (lineages 5 and 6) and the ancestral position of *M. canettii*. The animal lineages represent a monophyletic branch in the complex. The lineages in the grey oval are the so-called 'modern' lineages, as opposed to all the other lineages, which are called 'ancestral'.

reference technique. A worldwide database has been created that includes 7104 different spoligotypes corresponding to 58 187 clinical isolates from 102 countries (SITVITWEB; Demay *et al.*, 2012). It must be noted that isolates from developing countries, and particularly from African countries, are unfortunately not well enough represented in this database. Among the 7104 spoligotypes of this database, 24 represent the majority of the characterized clinical isolates. Particularly, the major MTB families represented are: Beijing, Central Asian (CAS), East African Indian (EAI), Haarlem (H), Latin American Mediterranean (LAM), T (badly defined) and X. These data suggest that some families are transmitted more efficiently than others. Different factors can explain this different potential, such as family-specific intrinsic characteristics and also the health conditions of the infected population. Interestingly, most of the major families described in the database belong to lineages identified as 'modern': Beijing belongs to lineage 2, CAS to lineage 3 and Haarlem, LAM, X and T to lineage 4 (Brudey *et al.*, 2006; Gagneux *et al.*, 2006). Therefore, these lineages might have a more elevated epidemiogenic potential. Moreover, these lineages have been generally associated with the recent transmission of TB and with

epidemics. This is true particularly for the Beijing family, which is ubiquitous and in the process of invading many countries, such as Vietnam (Nguyen *et al.*, 2012), and also for the LAM family, which is spreading in Africa (Godreuil *et al.*, 2007c; Groenheit *et al.*, 2011).

These observations have stimulated scientists to determine whether these lineages present specific virulence features. As MTB seems to evolve clonally, essentially by substitutions, deletions and duplications, it is thus plausible that some lineages might have acquired specific virulence and/or resistance features before spreading in the populations [see, for a review, Nicol & Wilkinson (2008)]. One of the most illustrative examples is that of the W-Beijing type. These strains have been predominant in many regions of East Asia. Moreover, their epidemic propagation is generally associated with multiple-antibiotic resistance (Agerton *et al.*, 1999; Anh *et al.*, 2000; Drobniewski *et al.*, 2002; Narvskaya *et al.*, 2002; Tracevska *et al.*, 2003; Johnson *et al.*, 2006). *In vivo* experiments in animals, particularly in rabbits and mice, have shown that these strains are highly virulent, with increased dissemination and earlier death of the infected animals (Manca *et al.*, 2001; Tsenova *et al.*, 2005). Overall, several *in vitro* studies have demonstrated

that the host response against modern and ancestral MTB lineages is different (Chakraborty *et al.*, 2013). Specifically, a more rapid disease progression and a more elevated transmission potential have been observed following infection by modern lineages (Portevin *et al.*, 2011; Reiling *et al.*, 2013; Chen *et al.*, 2014). Based on these epidemiological and experimental data, it seems clear that MTB has evolved towards an increased transmission and virulence potential. On the other hand, resistance to antibiotics does not seem to affect its transmission potential or its virulence, different from what would be expected (reviewed by Borrell & Gagneux, 2009).

An exemplary model of adaptation to humans

As TB could be an ancient human disease (Donoghue *et al.*, 2004; Gagneux, 2012), it is indispensable to study its history in parallel with that of humans in order to understand how this bacterium has evolved and the current TB epidemiology. Indeed, recent data have demonstrated that, contrary to what was thought, TB has been affecting humans long since before the development of agriculture and animal/plant domestication during the Neolithic transition. This bacterium might have emerged in humans about 70 000 years ago (Gagneux & Small, 2007; Comas *et al.*, 2013). Indeed, Roberts *et al.* (2009) have proposed that TB could predate modern humans, after the discovery in Turkey of a 500 000-year-old fossil of *Homo erectus* with typical TB bone lesions. These studies suggest, as described above, that contrary to the widespread hypothesis, MTB does not have an animal origin, but a human origin. This hypothesis is strengthened by the smaller size of the genome of *M. bovis* (60 000 bp smaller) and of other MTBC animal species compared with the human species (Cole *et al.*, 1998; Garnier *et al.*, 2003). MTB and animal species, like *M. bovis*, thus could share a common ancestor, and the transfer might have occurred from humans to animals at the moment of animal/plant domestication during the Neolithic period (Brosch *et al.*, 2002). Nevertheless, a recent analysis of *Mycobacterium* genomes taken from Peruvian skeletons of the pre-Columbian period suggests that sea mammals could play a role in the transmission of TB beyond the ocean. The data support the hypothesis of an introduction of MTBC into the American continent via the pinnipeds, followed by a human adaptation and a subsequent spread in the territory (Bos *et al.*, 2014).

According to Gutierrez *et al.* (2005), the common ancestor of all these MTBC species could have originated from *M. canettii* and the group of smooth tubercle bacilli from East Africa. These authors estimated, on the basis of the sequences of six housekeeping genes, an approximate age of three million years for this common ancestor, which they called *Mycobacterium prototuberculosis*. However, this dating has been contested by Smith (2006). It has to be noted that Africa is the only region that presents all seven MTBC lineages adapted to humans and thus the biggest genetic diversity in the world (Gagneux, 2012; Firdessa

et al., 2013). This situation totally mirrors that of modern humans. Indeed, *Homo sapiens* from Africa is considered to be the ancestor of modern humans and to contain the biggest genetic diversity (Tishkoff *et al.*, 2009). Finally, MTBC adapted to humans shows a phylo-geographical population structure with lineages that are more specifically associated with some human populations (Gagneux *et al.*, 2006). On these bases, the proposed scenario is that human MTBC could have originated from Africa and that it has already been infecting humans for thousands of years. The migrations of modern humans out of Africa and the increased population density during the Neolithic period could be at the origin of its expansion (Hershberg *et al.*, 2008; Gagneux, 2012; Comas *et al.*, 2013). Indeed, while four ancient lineages might have remained in Africa [i.e. the two *M. africanum* lineages, *M. canettii* and lineage 7, recently described by Firdessa *et al.* (2013)], the other lineages could have left Africa and spread, and the three modern lineages might have invaded Europe, India and China, possibly owing to the extraordinary population increase in these regions. Afterwards, human migrations, trading and human colonization of countries and continents could have contributed to their dispersion to become finally endemic everywhere in the world (Gagneux & Small, 2007; Hershberg *et al.*, 2008). This scenario was confirmed by the detection of the molecular signatures of the expansion of recent populations (less than 200 years) based on the study of minisatellites (Wirth *et al.*, 2008). These authors observed that these signatures were more pronounced in the European and Asiatic populations than in the African populations, supporting the 'out-of-and-back-to-Africa' scenario proposed by Hershberg *et al.* (2008).

It is obvious now that MTBC has evolved together with humans for a long time and that they have thus influenced reciprocally their evolution. Epidemiological data and the work by Comas *et al.* (2013) on the comparison of mitochondrial DNA from humans and from MTBC lineages suggest that the different lineages might have specifically adapted to the different human populations. Indeed, specific human genetic variants have been associated with different MTB lineages, clearly suggesting tight interactions between humans and the MTBC. These data indicate that, as for many other infectious diseases (Bañuls *et al.*, 2013), the long co-evolution of MTBC with humans had an effect on the biology and epidemiology of human TB. It is interesting to note that, currently, TB has all the features of a high-population-density disease, and also of a low-population-density disease. Indeed, its airborne transmission is characteristic of a disease transmitted in high-population-density areas. In parallel, its capacity to remain latent in humans for several decades, while still evolving, is typical of a disease transmitted in regions with low population density (Gagneux, 2012, 2013). This feature could be the result of a host-pathogen co-adaptation that might have allowed MTBC survival during the long period when inter-human transmission was rare and sporadic.

The consequence of this feature is that one-third of the human population is infected but asymptomatic, a phenomenal and worrying reservoir of this bacteria. For this reason, TB thrives in all ecosystems, from the most rural to the most urbanized. This duality is a terrible weapon and proof of its adaptation to the demographic history of humans.

Gagneux (2012) explains in his review that if a specific host–pathogen adaptation had taken place, changes in the genome of the bacterium interacting with the host immune system should be detected, particularly in genes encoding antigens. Classically, in host–pathogen systems, there is an arms race between pathogen and host immune system that normally results in a modification of the pathogen antigens to evade the host immune system (Dawkins & Krebs, 1979; Frank, 2002). A molecular evolution study carried out by Comas *et al.* (2010) showed that, as expected, the essential genes were evolutionarily conserved. However, surprisingly, the authors demonstrated that the MTBC epitopes recognized by human T cells were the most conserved genomic regions. To explain this specific pattern, they suggested that the immune responses elicited by these epitopes could be beneficial for the bacterium. In other words, instead of evading the host immune system, the MTBC uses the strategy of being recognized. This hypothesis is all the more plausible because the elicited immune response contributes to tissue destruction and to the formation of lung cavities, thus ultimately improving TB transmission (Rodrigo *et al.*, 1997). It is also supported by the finding that patients with TB/HIV, who have a very low level of CD4 T cells, tend to have fewer TB cavities (Kwan & Ernst, 2011). The hyper-conservation of these epitopes could thus be one of the key elements of successful MTBC propagation. Gagneux (2012) insists, nevertheless, that this does not exclude, of course, the possibility that the MTBC might present antigen variations in other genome regions.

Human demography can also explain the different evolution of virulence in modern and ancient lineages. As underlined by Gagneux (2012), in classical models of evolutionary biology, virulence is positively correlated with transmission, and the access to the largest possible number of susceptible hosts favours a strong virulence and a short latency period (Levin, 1996). MTB seems to have followed this model. Part of its evolution could have occurred during the hunter–gatherer period, when the human population density was still low. In this demographic context, MTB latency, followed by reactivation and disease several decades later, could have allowed access to new susceptible host cohorts, while at the same time escaping extinction caused by overly strong virulence (Blaser & Kirschner, 2007). On the other hand, the fast human expansion and the high population density in the overcrowded cities of the 18th and 19th centuries in Europe could have selected ‘less-cautious’ bacteria, because access to the susceptible hosts was not a limiting factor [see Gagneux (2012) for a review]. This scenario could explain

why modern lineages seem to be more virulent and are associated with a shorter latency phase than ancient lineages. These lineages thus show more efficient evolutionary success in terms of geographical propagation than the ancestral lineages.

From a more mechanistic point of view, the first human cells encountered by the bacterium are alveolar macrophages (Russell, 2011). MTBC bacteria are phagocytosed classically by these cells, but they have developed intracellular mechanisms to avoid destruction. These mechanisms allow the bacteria to survive and to multiply within macrophages. Experimental studies have shown that, compared with ancient strains, strains from modern lineages induce a significantly reduced inflammatory reaction in macrophages derived from human monocytes. This could be at the origin of their stronger virulence and shorter latency phase in animal models (Mitchison *et al.*, 1960; Reed *et al.*, 2004; Tsenova *et al.*, 2005; Portevin *et al.*, 2011). Concomitantly, a molecular epidemiology study carried out in humans living in Gambia demonstrated that there was no difference in transmission between modern and ancestral lineages, but that ‘contact’ people (i.e. people who slept in the same compound as index cases with active disease) were more susceptible to developing the active disease after infection by modern lineages (de Jong *et al.*, 2008). It is in this context that Gagneux (2012) suggested that the ‘modern’ strains have been evolving towards an increased virulence and a shorter latency phase in response to the phenomenal increase of the human population and thus of susceptible hosts.

We have discussed signatures that demonstrate MTBC adaptation to humans, but what about human adaptation to MTBC? Work in different host–pathogen systems has shown that if humans have influenced the evolution of micro-organisms, pathogens have also modulated and still modulate human evolution (Bañuls *et al.*, 2013). Concerning our model, as described before, Comas *et al.* (2013) have determined that the phylogeny of the complete genome of several MTBC strains mirrors that of human mitochondrial genomes. This pattern clearly suggests the co-evolution of humans with MTBC. Other studies also have highlighted associations between human genetic variants of immune system genes and some MTBC lineages (Caws *et al.*, 2008; Herb *et al.*, 2008; Intemann *et al.*, 2009; van Crevel *et al.*, 2009). Particularly, work carried out in Vietnam has shown that a specific SNP in Toll-like receptor 2, a key molecule for MTBC recognition by innate immunity, is associated with infection by the lineage 2 of MTBC (Caws *et al.*, 2008). Humans also show variations in TB susceptibility (Comstock, 1978), and many loci linked to susceptibility and resistance to TB have been identified (Casanova & Abel, 2002). However, a theoretical study shows that the time required for increasing the frequency of disease resistance loci in human populations is relatively long (more than 300 years) and specifically it is too long to have had a net effect on the

mortality associated with TB during the big epidemics, such as that of the second half of the 19th century, for example (Lipsitch & Sousa, 2002). We now know that TB has been infecting humans for thousand years and thus it is quite likely that it was during the hunter-gatherer period that low TB mortality emerged in the human population.

Conclusion

It seems obvious that human MTBC has imposed and still imposes a selection pressure on human populations, given the long co-evolution between MTBC and humans, the ability of the bacteria to remain latent in humans, the phenomenal proportion of asymptomatic carriers, the devastating epidemics and its increasing potential of resistance to antibiotics. The described epidemiological, evolutionary, physiological and molecular features and the history of these bacteria highlight their evolutionary success. However, according to Gagneux (2012), it is important to note that the low rate of active TB relative to the number of people with latent TB suggests that MTB is not a 'potent' pathogen. It is, nevertheless, very efficient in generating a secondary infection following active TB. In this context, Gagneux (2012) hypothesized that infection by MTB could be beneficial for some individuals who never developed the active disease. The constant, but low, level of immune stimulation generated by the latent infection could protect against other diseases (Comas *et al.*, 2013). Accordingly, the BCG vaccine, based on a strain of *M. bovis* that has lost its virulence, is a potential immune adjuvant used to treat some cancers. It must, however, be stressed that owing to the mortality and morbidity caused by TB, it would be inappropriate to characterize MTB as a commensal or a symbiont (Gagneux, 2012).

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