Steps towards the discovery of *Mycobacterium tuberculosis* by Robert Koch, 1882

E. Cambau1,2,3, † and M. Drancourt4

1) APHP, Service de Bactériologie, Hôpitaux universitaires Lariboisière-Saint Louis, 2) Université Paris Diderot, EA3964, 3) Centre national de référence des mycobactéries et résistance des mycobactéries aux antituberculeux, Paris, France and 4) Méditerranée Infection, Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD198, Inserm 1095, Marseille, France

Abstract

Palaeomicrobiology has detected the tuberculosis agent in animal and human skeletons that are thousands of years old. The German doctor Robert Koch was the first microbiologist to report in 1882 the successful isolation of the causative agent of tuberculosis, named 1 year later as *Mycobacterium tuberculosis*. This immense discovery, however, was not made from scratch, but involved the combining of previous scientific knowledge, chiefly the previous demonstration by the French doctor Jean-Antoine Villemin that tuberculosis was a transmissible disease, and two innovations—a new staining procedure that allowed R. Koch to consistently observe the new organism in tuberculous lesions, and use of a solidified, serum-based medium instead of broths for the culture. These innovations allowed R. Koch not only to isolate *M. tuberculosis* from animal and patient specimens for the first time, but also to reproduce the disease in experimentally inoculated guinea pigs. It is thanks to R. Koch that one of the most lethal diseases in human history could be diagnosed, could be treated and cured after the discovery of streptomycin 65 years later, and could be efficiently prevented by isolation of cases. His microbiological innovations are now being renewed with molecular and improved culture-based detection being the twenty-first century weapons in the fight against this disease, which remains a major killer.

Keywords: Acid-fast bacilli, cultures, *Mycobacterium tuberculosis*, palaeomicrobiology, staining

Article published online: 22 January 2014

*Clin Microbiol Infect* 2014; 20: 196–201

Corresponding author: M. Drancourt, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Faculté de Médecine, 27, Boulevard Jean Moulin, Marseille Cedex 5, France

E-mail: michel.drancourt@univ-amu.fr

†E. Cambauon—on behalf of ESGMYC (Study group of ESCMID on mycobacterial infections).

From Ancient Times to the Discovery of the Tubercle Bacillus

Although mycobacteria were supposed to exist for about 150 million years [1], *Mycobacterium tuberculosis* is a young species, aged about 150 000 years [2]. The disease itself, tuberculosis, has been known for centuries [3]. Mummies from the Egyptian pre-dynastic era and the Peruvian pre-Columbian era show typical vertebral lesions [4,5]. The antiquity of tuberculosis is attested by palaeomicrobiology, which provides a demonstrative framework to assess the clinical descriptions of doctors and patients through the centuries [6–8]. The first weak evidence for tuberculosis in humans relies on lesions compatible with bone tuberculosis in a 500 000-year-old skull in Turkey [9], the first undisputed evidence of *M. tuberculosis* was obtained by PCR sequencing and detection of mycobacterial lipids in bone lesions of a 17 000-year-old bison found in Wyoming, USA [10,11]. Much work was then done to attest to the presence of human tuberculosis in several burial sites in Europe [7,12] and in Asia [13]. The oldest evidence for human tuberculosis was found in a Neolithic infant and woman in a 9000-year-old settlement in the Eastern Mediterranean [13]. The vast majority of studies detecting *M. tuberculosis* DNA sequences have used bone as the material on which to base the diagnosis, whereas a few works used lung tissue [14,15] or other soft tissues [16].
The literature is full of tuberculosis descriptions. This frequent disease was named as \textit{schachepheph} in the Old Testament [3], \textit{phthisis} as described by Hippocrates in Greek literature, \textit{consumptio} in Latin reports from the Roman Cicerone and \textit{consumption} in many occidental myths of the nineteenth century (such as \textit{La Dame aux Camélles} and \textit{La Traviata}) and in the deaths of Chopin and Chekov. Johann Lukas Schönelein eventually unified the nosology and proposed the name 'tuberculosis' in 1834 due to the presence of tubercles in all forms of the disease.

Indeed, the discovery of \textit{M. tuberculosis} by Robert Koch (1843–1910) came into a framework of previous popular and scientific knowledge regarding tuberculosis. Hippocrates considered tuberculosis to be hereditary. Galen (131–201) first suspected that it could be transmitted, then Girolamo Francastoro (1483–1553) showed that some diseases could be transmitted through 'particles' by direct or indirect contact between humans. Some people, such as the English physician Benjamin Marten (1690–1752), already hypothesized in 1720 that consumptions could be caused by 'wonderfully minute living creatures', which could lead to the lesions that are symptomatic of the disease, thereby expressing the theory of 'contagious living fluid' [17]. He went on to state that 'it may be therefore very likely that by a habitual lying in the same bed with a consumptive patient, constantly eating and drinking with him, or by very frequently conversing so nearly as to draw in part of the breath he emits from the lungs, a consumption may be caught by a sound person … I imagine that slightly conversing with consumptive patients is seldom or never sufficient to catch the disease.' Marten's epidemiological insight was prescient, but was not really helpful in the absence of direct observation or experimental data. Although Leeuwenhoek had reported seeing bacteria in 1676, he had not believed that his 'little animals' caused disease. Koch's discovery was made within the framework of the theory of germs that was progressively elaborated in Europe during the first half of the nineteenth century.

\textbf{Tuberculosis: Dispute on the Unicity of a Disease}

Clinical signs, symptoms and lethality (50% of the cases) attributed to tuberculosis were more precisely described during the nineteenth century. Whether typical Pott spondylitis was related to lung consumption (Hammurabi 1950 bc) and scrofula (described by Aristotle, 384–322 bc) was discussed for centuries. Finally, Sylvius de la Boë of Amsterdam (1617–1655) then René Théophile Hyacinthe Laennec (1781–1826), agreed that they were several clinical and anato-mo-pathological forms of the same disease. Typical clinical signs of tuberculosis had already been reported earlier by a Roman physician (500 bc) with typical coughing associated with fever during the night with sweats. The military tuberculosis, as described by Thomas Willis (1621–1675), seemed to be more frequent at that time than it is now, probably because it was the era before bacillus Calmette–Guérin (BCG) vaccination.

A further step was made when human tuberculosis was related to the animal disease, especially that observed in cattle. Jacob Henle (1809–1885) observed the transmission from cat to human and inoculated human specimens to rabbits. He opened the field to Robert Koch, who stained bacilli from human lesions as well as those from animal lesions from cows, pigs, hens, monkeys, rabbits and guinea pigs. It was acknowledged that \textit{M. tuberculosis} infection causes various forms of diseases, depending on the affected organ and tissue. Pulmonary tuberculosis was less prevalent than it is today because the oral route and airborne transmission were probably equally successful.

\textbf{Jean-Antoine Villemin Demonstrated the Transmissibility of the Disease}

The transmission of 'consumption' from a coughing patient to a healthy person was just unbelievable for people living before the nineteenth century because tuberculosis was considered to be inherited or congenital or spontaneous. Decades were needed to show that tuberculosis was transmitted from human to human, from human to animal or from animal to human. Interestingly, the first sentence of the seminal paper by Koch acknowledges previous work by Jean-Antoine Villemin (1827–1892), a French army doctor, who first demonstrated the transmissibility of tuberculosis [18] (Fig. 1).

In fact, Jean-Antoine Villemin, successfully transmitted tuberculosis from patient to rabbit, from cow to rabbit and from rabbit to rabbit as early as 1865 [19,20]. These seminal data were further confirmed by John Sanderson and John Simon, appointed by the British Government to investigate that topic [21]. Tappeiner was the first to describe a guinea pig model with bacillus inhalation [21].

\textbf{Robert Koch's Discovery of the Tubercle Bacilli}

On 24 March 1882, the German doctor Robert Koch communicated to the Berlin Society of Physiology that he had discovered the microorganism responsible for the deadly...
pulmonary tuberculosis, named *Tuberkelvirus* in his seminal publication made 2 weeks later [18] (Fig. 1). These first isolates of the aetiological agent are now displayed at the Hunterian Museum at the Royal College of Surgeons of England, London, UK [22]. The tuberculosis agent was named as *Mycobacterium tuberculosis* in 1883 [23]. Further molecular analysis of these very first isolates confirmed the identification of *M. tuberculosis* and indicated that Koch’s isolates belong to the ‘modern’ lineage of *M. tuberculosis*.

Staining tuberculosis bacteria was a first, decisive innovation made by R. Koch and his team. As stated by R. Koch himself, the goal was to demonstrate the presence of ‘foreign parasitic structures… indicative of the causal agent’. However, R. Koch stated that ‘the staining methods which have been so useful in the demonstration of pathogenic micro-organisms have been unsuccessful here’ [18]. Indeed, R. Koch observed that the alcohol-methylene blue staining developed by Karl Weigert in 1875 [24] did not easily stain in tuberculous lesions. Tubercle bacilli were stained only after a long 24-hour contact with Weigert’s blue and further contact with KOH, before being counterstained by vesuvine, exactly as the leprosy bacillus [25].

R. Koch also noticed that heating the preparation at 40°C greatly reduced the time required for methylene blue staining to only 1 hour. Using this staining, R. Koch reported that the animal tissue structures stained brown whereas tubercle bacteria stained a beautiful blue, contrary to any other bacteria, which stained brown [17]. R. Koch insisted that the contrast between brown-coloured tissues and blue-coloured tubercle bacteria rendered them easily detected by microscopy. Paul Ehrlich and then R. Koch, further modified the staining protocol (notably by heating stained slides), and the technique was finalized by Ziehl and Neelsen in 1885 [25]. Using this new staining method, R. Koch reported that the tubercle bacteria are rod-shaped organisms appearing as bacilli and looking very similar to the leprosy bacilli. He noticed that bacilli appeared as grouped within the lesions, either intracellularly or extracellularly.

At that point, R. Koch noticed that the repeated observation of tubercle bacilli was not proof for this organism being the aetiological agent of tuberculosis. However, these microscopic observations convinced him that the microbial hypothesis of tuberculosis was right and paved the way to tentative isolation of the bacilli in pure culture.

R. Koch then embarked on isolating the tuberculosis bacillus. Development of solid culture media instead of the culture broths used at that time was a second decisive innovation. In fact, R. Koch had already published descriptions of such solid media before he used them to isolate *M. tuberculosis* [26]. He simply used solidified cow or sheep serum, with sterilization being obtained by repeated, short courses of heating at 58°C, i.e. a procedure previously reported by Tyndall. Final, prolonged heating at 65°C then solidified the medium, which was poured in
slanted tubes so as to increase the surface available for inoculation. This also permitted the tube to be kept for weeks without contamination, although it contained the rich medium necessary for *M. tuberculosis* growth. After inoculation, tubes were incubated at 37–38°C and growing colonies were detected by the naked eye. Initial experiments were made by inoculating experimentally infected guinea pig lung tissues and colonies were observed after 10–14 days. Further, a total of 15 pure cultures were obtained from four experimentally infected guinea pigs, four from bovine tuberculosis and seven from patients. Interestingly, R. Koch noticed that detection of growing colonies using 30× to 40× magnification allowed detection of colonies by the end of the first week [18].

This great discovery gave Robert Koch the Nobel Prize of Medicine in 1905 ‘for his investigations and discoveries in relation to tuberculosis’ (Nobel Foundation website) and a worldwide reputation that is still strong after more than 130 years.

**Koch’s Postulates: to Demonstrate the Cause of the Disease being an Infectious Agent**

R. Koch was not only able to isolate and then culture this new microorganism from sputum, but also to demonstrate that the disease was due to an external cause, an ‘infectious agent’. Three steps were necessary to achieve this demonstration. First, the infectious agent was observed in lesions typical of the disease but not in other lesions or in normal tissues. Second, the infectious agent was cultured *in vitro* outside the infected human or animal. Third, the infectious agent, after growing *in vitro*, was reintroduced into a non-infected animal and led to typical tuberculosis lesions. The disease was reproduced *in vivo* with the same features by using specimens either from a tuberculosis patient or an animal or the culture: pus, sputum and lung tissues were inoculated to guinea pigs, rabbits and cats; cultures were inoculated to guinea pigs. The same lesions appeared in 2 weeks and death was observed in 8 weeks. All inoculated animals showed disease but none of the non-inoculated animals showed disease. However, non-inoculated guinea pigs became infected when living in the same room as inoculated animals. This showed the airborne route of transmission of the infectious agent.

**From Discovery to Eradication**

At the time when the bacillus was discovered, in early 1882, one out of seven world inhabitants was dying from tuberculosis [18], which would be about one billion deaths per year in 2013 if nothing had happened since the Koch postulates.

The demonstration that (i) a bacterium, *Mycobacterium tuberculosis*, was responsible for the tuberculosis disease, that (ii) this bacterium was transmitted from human to human, and that (iii) this disease can only be acquired through transmission from a patient or animal with tuberculosis, helped in stopping the spread of the disease. This was the beginning of a trend to its eradication. Robert Philip launched in 1887 a large programme of active prevention of tuberculosis transmission in Edinburgh [27,28].

When the tuberculin, a protein that was also produced by R. Koch, was extracted from large cultures of the bacillus by Calmette, it was first used as a therapy, ‘tuberculisation’, which failed, and second for the diagnosis of tuberculosis with the well-known tuberculin skin test. The description of the intra-dermal reaction by Charles Mantoux led to an understanding of much of the immunology and physiopathology of tuberculosis infection [29]. By reading his report to the Société de Biologie, one may consider that very little has changed from 1910 to 2013 considering the results of interferon-γ release assay testing [30].

The same Leon Charles Albert Calmette (1863–1933), together with Camille Guérin (1872–1961), sub-cultured more than 200 times, between 1908 and 1921, a peculiar strain called *Mycobacterium bovis*, and showed that the resulting bacterium had lost its virulence in the guinea pig model [31,32]. The era of vaccination was started. BCG immunization greatly reduced the number of meningitis cases in infants and the incidence of miliary forms, even in adults [33]. It is now known, a hundred years later, that *M. bovis* BCG is a variant harbouring more than 12 DNA deletions with regard to *M. tuberculosis* [34].

Finally, the discovery of streptomycin in 1943 by Albert Schatz, directed for this PhD thesis by Selman Waksman (Nobel Prize 1952), ended the great period of discoveries concerning tuberculosis [35,36]. Whether the genomic deciphering of the tubercle bacillus will help to eradicate tuberculosis in the world during the twenty-first century is still a hope.

**R. Koch’s Lesson**

R. Koch paved the way to culture-based diagnosis of tuberculosis. In fact, the results he obtained have not been surpassed by further developments in culture media that aimed to abandon serum in order to use more easily available ingredients such as egg and further synthetic media. This may have been a mistake because the time to detection routinely
achieved is usually longer than the 1 week reported by R. Koch. The chance observation that sheep-blood agar supported the growth of *M. tuberculosis* [37,38] led to a renewed interest in media containing sheep blood [39,40] and recently, a sheep serum-agar medium was reported for testing *M. tuberculosis* antibiotic susceptibility [40]. We recommend that the basic R. Koch medium is not forgotten. Strategies coupling rapidly growing medium, early detection and rapid identification should be targeted by the mycobacteriologists of the twenty-first century [41,42]. The new weapon for fighting tuberculosis is currently molecular detection [43], and it brings the hope of eradicating the disease in the near future.

**Transparency Declaration**

The authors declare no conflicts of interest.

**References**

