Historical Review

100th anniversary of Robert Koch’s Nobel Prize for the discovery of the tubercle bacillus

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The year 2005 marks the 100th anniversary of Robert Koch’s Nobel Prize. Here, we describe the scientific career of Robert Koch, the discoverer of the etiologic agent of tuberculosis but also of those of anthrax, cholera and wound infections. Equally important, Koch developed the methodologies and concepts that made medical microbiology a scientific discipline. Despite great efforts, however, Koch failed to conquer tuberculosis, which still causes enormous health problems worldwide 100 years after his Nobel award.

Introduction

One hundred years ago, Robert Koch (1843–1910; Figure 1) was honoured with the Nobel Prize for his groundbreaking work on tuberculosis. The laudation did not mention his discovery of the etiologic agent of tuberculosis specifically but rather emphasized the achievements of Koch in the fight against this threat. Accordingly, in his award lecture ‘On the current status of the fight against tuberculosis’, Koch focussed on epidemiologic and hygienic measures for reducing morbidity and mortality from tuberculosis [1]. This re-emphasis on more conventional public health strategies was in contrast to his original hope that implementation of the new discipline of medical bacteriology would promote rational intervention strategies against tuberculosis (Box 1). In his groundbreaking lecture ‘On the etiology of tuberculosis’ in 1882, Koch proclaimed that his findings brought to an end the prevalent notion that tuberculosis was merely a consequence of social conditions, and that novel, more efficacious intervention strategies should take into account the infectious nature of this disease [2]. Accordingly, at this time he was quite optimistic that eradication of tuberculosis would be feasible. Unfortunately, this was an overly optimistic view and tuberculosis remains one of the major global health threats of today.

Each year 9 million new cases of tuberculosis are recorded, of whom 2 million die. Although chemotherapy and vaccination are available, the disease has not been conquered [3]. The vaccine Bacille-Calmette-Guérin (BCG) only prevents childhood tuberculosis and largely fails to protect efficaciously against the most prevalent form, pulmonary tuberculosis in adults. Chemotherapy is highly effective but requires many months of treatment with at least three different drugs, hampering compliance. As a corollary, multi-drug-resistant (MDR) strains are on the rise. The situation is further exacerbated by the emergence of the acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV). Coinfection with HIV and Mycobacterium tuberculosis dramatically increases the risk of developing tuberculosis by a factor of several hundred.

Overture

When Koch embarked on bacteriological research, the concept that certain diseases were contagious was not

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Box 1. Major achievements of Robert Koch

**Discovery of bacterial pathogens**
- 1876: Discovery of the etiologic agent of anthrax, *Bacillus anthracis*.

**General methods**
- 1876–1882: Development of solid culture, staining and disinfection methods, and the introduction of microphotography for microbiological research.
- 1890–1891: Discovery of tuberculin (PPD) for diagnosis of tuberculosis.
- 1883–1892: Development of public health measures and successful application for control of cholera.

**Tropical diseases**
- 1890–1906: Life cycle of plasmodium malaria; description of increasing host resistance against malaria with age; broad-scale quinine treatment of high risk individuals in malaria endemic areas; prevention and therapy of sleeping sickness.

completely new. Infection, however, was mainly thought to occur by transfer of disease through a miasma that was generated by degradative and fermentative processes. The most important step towards the new discipline of bacteriology was the work of Louis Pasteur (1822–1895) in Paris, who had proven that life is not generated spontaneously and that microorganisms cause fermentation and putrefaction [4]. From the medical side, Philip Ignaz Semmelweis (1818–1868) in Austria had described impressively the infectious nature of puerperal fever and developed simple but effective methods for its prevention, which unfortunately were stubbornly ignored by his colleagues [5]. Equally striking were the studies of the British surgeon Joseph Lister (1827–1912), who had developed successful methods for the treatment and prevention of wound infections using carbolic acid [6].

When Koch started to tackle his first bacterial target, *Bacillus anthracis*, in 1873, he could build on some hints pointing to the infectious etiology of anthrax and the existence of a causative agent. Most significantly, Casimir Joseph Davaine (1812–1882) had already discovered rod-like microbes in the blood of animals suffering from anthrax, which he claimed to be the causative agents.

Koch attempted to fulfill the major prerequisites for defining a contagious disease proposed by his former mentor Jacob Henle (1809–1885) [7]: these include the presence of a living contagion in the diseased body, the isolation of this contagion from the diseased body and the transfer of the same disease by the contagion (Box 2).

He established the conditions for spore formation and bacterial germination and replication, developed solid suitable medium for pure culture and successfully infected experimental animals with the anthrax bacillus. As a complete unknown, who had to perform his experiments in solitude in his private small laboratory outside of the university, Koch was eager to receive feedback on and confirmation of his work. He received an extremely positive response from botanist Ferdinand Cohn (1828–1898) at the Institute for Plant Physiology at Breslau (now Wroclaw) University. The paper was published in 1876 under the title 'The etiology of anthrax based on the development of *Bacillus anthracis*’ [8] (Figure 2).

Before turning his attention to tuberculosis, Koch approached one other infectious disease of great importance, namely wound infection. The Prussian-French war of 1870–1871, with its thousands of wounded soldiers, had drawn particular attention of the public, policy makers and medical establishment to these diseases, which were responsible for the lethal outcome of many otherwise minor injuries. Koch revealed that several different bacterial species could be isolated from wounds and that these bacteria caused a wide spectrum of clinical

Box 2. The Koch-Henle postulates for the definition of microbial pathogens

**General definition of Koch’s postulates by Friedrich Loeffler for diphtheria in 1884 [23] and fulfilled in a paradigmatic way by Robert Koch for tuberculosis in 1882 [2].**

Loeffler’s general formulation of the postulates (1884):

‘... the following three postulates need to be fulfilled to demonstrate the infectious nature of the disease:
(i) The organism must be present consistently in characteristic shape and arrangement in the diseased tissue.
(ii) The organism responsible for the disease must be isolated and grown in pure culture.
(iii) The pure culture must be able to induce the disease in experimental animals.

Koch’s paradigmatic fulfillment of the postulates for tuberculosis (1882):

**The first postulate**

‘... at all sites, where the tuberculous process was fresh and progressed rapidly, bacilli were found in abundance; they formed small and densely packed groups often within host cells. ... Aside from this, numerous free bacilli could be detected.’

**The second postulate**

‘Serum from cattle or sheep blood ... is heated at 58°C for 1 h daily for 6 days. This ... results in sterilization of the serum. It is then heated at 65°C for several hours after which it solidifies. After this treatment, the serum is ... fully translucent. To achieve a large surface for culture ... the serum is left in slanted position. ... single tuberculous nodules or particles thereof ... are prepared from infected lung tissue and immediately ... transferred to the serum culture. ... colonies become visible as small dry spots in the second week after inoculation, generally not before the 10th day’.

**The third postulate**

‘Of 6 guinea pigs which had been kept in a single cage after purchase, 4 animals were inoculated intraperitoneally with a bacterial culture derived from a human lung with miliary tuberculosis and cultured for 55 days through 5 passages. Two animals were left untreated. In the infected animals, inguinal lymph nodes became swollen after 14 days, at the site of injection, lesions developed and the animals lost weight. After 32 days, one of the vaccinated animals died. After 35 days, the other animals were killed. All vaccinated animals ... suffered from severe tuberculosis in spleen, liver and lungs; the inguinal lymph nodes were markedly swollen and caseous, ... the 2 untreated animals showed no signs of tuberculosis in lung, liver and spleen.’

**Conclusion**

‘All these factors together allow me to conclude that the bacilli present in the tuberculous lesions do not only accompany tuberculosis, but rather cause it. These bacilli are the true agents of tuberculosis.’
syndromes in experimental animals including abscesses, sepsis, tissue necrosis and fever. The work of Koch appeared in 1878 in the booklet ‘Investigations about the etiology of wound infections’ [9].

**The first act: Koch’s greatest success**

Koch gradually became the most famous proponent of the new field of medical bacteriology, at least in German-speaking countries, and soon was offered the directorship of the bacteriological laboratory at the Imperial Health Office in Berlin, which he accepted in 1880. He immediately took advantage of the new opportunities in this well-established institute and developed all the methodologies required for bacteriological work, including staining methods, solid culture media, disinfection methods, incubation boxes, experimental animal studies and, last but not least, microscopy and microphotography. Notably, he developed microphotography as a tool for objective presentation of his findings independent of a witness (Figure 2). Thus far, convincing evidence for the presence of microbial organisms depended on confirmation by a reliable attester that drawings of microbes reflected nature as closely as possible. With the introduction of microphotography and its further development, Koch now had at hand a tool that enabled him to document his findings independent of time and place of discovery.

After a year of hard work in Berlin, Koch was ready to start with his most challenging task, tuberculosis.

Figure 2. Original drawing of *Bacillus anthracis* by Koch and Cohn. Insert: Photomicrography of *B. anthracis* by Koch (1876). Figures reproduced from Ref. [22].
Scattered reports hinting towards the contagious nature of tuberculosis already existed. Notably, Jean-Antoine Villemin (1827–1892) in France, and Julius Cohnheim (1834–1884) in Germany, had transferred the disease with clinical material to experimental animals. Moreover, at about the same time as Koch performed his work, Paul Clemens von Baumgarten (1848–1928) in Königsberg (now Kaliningrad) identified the agent causing tuberculosis [10]. Baumgarten, however, had neither succeeded in culturing the microorganisms nor in transferring disease to experimental animals.

Koch described the etiology of tuberculosis for the first time in a scientific lecture given at the Physiological Society in Berlin on the evening of 24 March 1882 (Figure 3). In recognition of the importance of this day for tuberculosis control, the World Health Organization announced 24 March as the ‘World Tuberculosis Day’. Only three weeks after his lecture – on 10 April 1882 – the paper ‘The etiology of tuberculosis’ was published in the prestigious journal *Berliner Klinische Wochenschrift* (BKW, Berlin Medical Weekly) [2]. In his paper, Koch defined tuberculosis as an infectious disease beyond doubt by fulfilling the Koch-Henle postulates in a paradigmatic way, which is notable because tuberculosis is one of the slowest growing and most difficult to stain microbes.

Towards the end of the 19th century, tuberculosis was the major health threat in Europe, hitting capitals and larger cities the hardest. Virtually all adults were infected with *Mycobacterium tuberculosis* (Otto Naegeli, 1871–1938) [11]. In Berlin, tuberculosis was the number

![Figure 3. Original drawing of tubercle bacilli within infected tissue by Koch, which accompanied his groundbreaking report on the etiology of tuberculosis. Figure reproduced from Ref. [22].](image)
one killer. Of all deaths, > 12% – the death of every third newborn and of almost every second adult aged 25–40 – were caused by tuberculosis.

**A new concept**

It probably took such a devastating disease and such a careful piece of work to push forward the new concept that exogenous agents transmitted from person to person caused the disease. The prevailing concept in medicine at the time, propagated by Rudolf Virchow (1821–1902), pathologist at the Charité in Berlin, rather proposed that all forms of disease arose from the malfunctioning of our own cells [12]. No room was given for exogenous causative agents. It was probably because of this controversy that Robert Koch was not invited to present his work to the most prestigious Berlin Medical Society, which was strongly influenced by Virchow, but rather was asked to deliver his lecture at the much smaller Berlin Physiological Society. Robert Koch was the one who had come up with a new concept, therefore, he had to provide evidence. To prove his ideas, he followed the Koch-Henle postulates [7] (see Box 2), as described in the subsequent sections.

**The first postulate**

Fulfilment of the first postulate for tuberculosis already posed unexpected technical difficulties. As is well known nowadays, tubercle bacilli are difficult to stain because of their robust wax-rich cell wall. They even resist harsh acid treatment, thus they are known as ‘acid fast bacilli’, a characteristic that is exploited for the specific staining of mycobacteria in diagnostic laboratories of today [the Ziehl-Neelsen stain by Franz Ziehl (1857–1926) and Friedrich K.A. Neelsen (1854–1894)].

In his attempts to reveal the tuberculosis agents, Koch originally employed a staining procedure using methylene blue, which required as much as one day at room temperature. Later, Koch noted that the incubation time could be shortened significantly by heating samples to 40°C during staining. Subsequently, samples were counterstained with brown vesuvin resulting in a dark blue staining of the tubercle bacilli versus the host tissue, which appeared brown in colour. Paul Ehrlich, who attended Koch’s lecture, improved this tedious staining procedure by adding aniline to improve the methylene blue staining and fuchsin for counterstaining so that the bacteria appeared bright red [13]. Koch identified stained tubercle bacilli in tissue, both inside and outside host cells (Figure 3). He also described the formation of multinucleated giant cells by the fusion of several macrophages and that these cells served as preferred habitat for the bacteria in paucibacillary conditions. Consistent identification of tubercle bacilli in affected tissues, both of patients and experimentally infected animals, fulfilled the first postulate unequivocally (Figure 3).

**The second postulate**

The second postulate, like the first postulate, also caused some major difficulties due to the slow growth of *M. tuberculosis*. For this purpose, Koch had to develop a special medium (see Box 2). After inoculating the medium with carefully selected samples from experimental animals or patient material, Koch had to wait patiently. After 10 days, the first signs of growth appeared as dry spots. After several transfers to new medium, larger colonies developed, which could be used for further analyses. Bacilli were isolated from a total of 33 cases, including patients suffering from miliary tuberculosis, caseous bronchitis and pneumonia, brain tuberculosis, intestinal tuberculosis, scrofulosis and joint tuberculosis, as well as from 13 cattle with bovine tuberculosis. The successful isolation and culture of bacteria fulfilled the second postulate unequivocally.

**The third postulate**

Large numbers of guinea pigs and rabbits, as well as some other animal species, were infected with tuberculous material from patients, diseased animals or with isolated cultures. Koch stressed that he had never found tuberculosis in newly purchased guinea pigs, although some animals developed tuberculosis sporadically within 3–4 months of housing together with infected animals. These naturally infected guinea pigs suffered consistently from bronchial tuberculosis because they had acquired the pathogens via aerosols. By contrast, for experimental infections, Koch generally injected the animals intraperitoneally and thus inguinal lymph nodes were the primary site of mycobacterial growth. Successful infection with isolated bacilli fulfilled the third postulate unequivocally.

**Intermezzo: the discovery of *Vibrio cholerae* and strategies towards cholera control**

Subsequent years created new challenges for Koch. Asiatic cholera threatened to attack Europe and the assumption was that this disease was caused by a microbrial pathogen. Cholera had been endemic in India for a long time but absent from Europe until the beginning of the 19th century. Between 1820 and 1880, several major waves of cholera had reached Africa. When cholera rampaged in Egypt in 1883, European governments feared it would conquer Europe. Both France and Germany sent their best bacteriologists to Egypt. Louis Pasteur was already too old to embark on this journey but his close coworkers, Emile Roux (1853–1933) and Louis Th Guillier (1856–1883) participated in the French team led by Isidore Straus (1845–1896). The German government asked Koch to lead their team. Together with his coworkers, Georg Gaffky (1850–1917) and Bernhard Fischer (1852–1915), Koch travelled to Egypt in 1883. The cholera bacillus survives in water pools for long times, causing periodic outbreaks after ingestion of contaminated water. The French researchers focused their investigations on material from cholera victims and, tragically, Th Guillier became infected and ultimately died. The German team succeeded rapidly in obtaining pure cultures of *Vibrio cholerae* from environmental and patient samples [14]. The success of the team was based principally on the use of solid media developed by Koch, which enabled the rapid isolation of pure cultures from highly contaminated specimens, whereas the French researchers still relied on liquid culture media. Thus, the first and second Koch postulates were fulfilled for cholera. Soon after Koch’s return to Berlin in 1884, a
cholera outbreak in Europe enabled Koch to fulfil the third postulate by infecting guinea pigs orally [15]. He succeeded by prior neutralization of the acidic gastric milieu with alkaline solution and the animals subsequently died of a cholera-like disease. Koch introduced hygienic measures, notably water-purification systems, and epidemiology to public health control. Ultimately, this helped to reduce the devastating consequences of the cholera outbreak in Hamburg, Germany, in 1892. This was the breakthrough of the concept that cholera has a bacterial etiology. Without doubt the discovery of *V. cholerae*, the elucidation of the epidemiology of cholera and the application to public health measures by Robert Koch is a success story of its own, which is sometimes forgotten in the light of his exceptional work on tuberculosis.

**The second act: Koch's greatest failure**

In 1890, the 10th International Congress of Medicine took place in Berlin with >5000 participants from 40 different countries. One of the keynote lectures was given by Koch. At the end of his talk, Koch made a thrilling announcement: he claimed he had discovered compounds that inhibited the growth of tubercle bacilli in guinea pigs, when given both pre- and post-exposure (see Box 3). This announcement evoked an enormous response, not only in the scientific community but also amongst laypeople. The perceived 'world famous' founder of medical bacteriology and the 'discoverer of *M. tuberculosis*', a scientist of highest reputation, had found a cure for the major health threat on Earth! Koch's paper was published in the proceedings of the congress [16]. The excitement of the medical world about this discovery is revealed by numerous reports on the work of Koch in *The Lancet*, *British Medical Journal* or *Scientific American* between 1890 and 1891. Subsequently, a series of three consecutive papers on tuberculin, as this remedy was soon termed, appeared in the prestigious *Deutsche Medizinische Wochenschrift* (DMW, German Medical Weekly).

The first paper of this series described several clinical studies with tuberculin but still failed to reveal the identity of the compound [17] (see Box 3). Koch showed that tuberculosis patients and healthy controls reacted differently to local injection of low doses of the remedy. Although patients developed specific symptoms at the site of injection, healthy individuals did not show any reaction to these low doses. Koch immediately envisaged the underlying mechanisms in foreseeing that the material did not act on the bacilli directly but rather by stimulating host cells and clearly foresaw the diagnostic value of his remedy. This was the birth of the delayed-type hypersensitivity (DTH) test, which is still in use today for diagnosing *M. tuberculosis* infected individuals. Koch was not only the discoverer but also one of the first to develop a positive tuberculin test.

The next paper revealed the identity of the material as a glycerol extract of liquid cultures of tubercle bacilli [18]. Here, Koch also gave a precise description of the differential reactions of healthy and infected guinea pigs to challenge with *M. tuberculosis*. The reaction of the already infected guinea pigs was far more severe, resulting in necrotizing lesions. This adverse reaction towards secondary challenge infection was later called the 'Koch phenomenon'.

The third and final paper described the preparation and partial purification of tuberculin in greater detail and thus served as the first description of the preparation of partially purified derivative (PPD) of tuberculin [19]. It also contained a description of the reactions towards this improved preparation of several healthy volunteers.

In the meantime, clinical trials on tuberculin as a therapeutic vaccine had been initiated and the official report on these clinical trials was published in 1891 [20]. This extensive report described the responses of a total of 1061 patients with tuberculosis of internal organs and of 708 patients with tuberculosis of external tissues, who had all been treated with tuberculin. These trials revealed that only a few individuals were cured, a success rate within statistical variation and, hence, no different from that in untreated patients. Accordingly, the report concluded that, although tuberculin was valuable for the diagnosis of tuberculosis, results for the treatment of tuberculosis were disappointing.

In parallel, the Medical Community in Berlin held numerous meetings on tuberculin treatment of tuberculosis. Although euphoria prevailed during the early phases, soon the opinion changed. In a meeting in January 1891, Virchow, who had analyzed carefully a large number of autopsy material, described the double-edged nature of the treatment [21]. He provided evidence that tuberculous granulomas dissolved under tuberculin treatment; on the one hand, allowing better access of immigrant blood cells to the bacteria and, on the other hand, facilitating bacterial dissemination to other organs.

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**Box 3. Koch’s greatest failure: a remedy against tuberculosis, which acts as pre- and post-exposure vaccine, was described in a series of four papers [16–19], which appeared between 1890 and 1891**

**First claim of a remedy (1890)**

‘... guinea pigs, which are well known to be highly susceptible to tuberculosis, no longer respond to infection with tubercle bacilli once they had been pretreated with such substances. Moreover, in guinea pigs that suffered from severe tuberculosis, the disease process could be brought to an end without harming the animals’ [16].

**Description of the underlying mechanism (1890)**

‘The most important feature of this remedy is its specific action on tuberculous processes of any kind. ... The material does not kill the bacilli in the tissue directly, but instead the tissue containing tubercle bacilli is affected by this treatment ...’ [17].

**Diagnostic value of the remedy (1891)**

‘... the substance will become an indispensable diagnostic measure in the future. It will enable us to diagnose questionable cases of early phthisis even when we fail to detect bacilli ...’ [18].

**Description of the remedy (1891)**

‘The substance, with which the new treatment against tuberculosis is being performed, is a glycerol extract of pure culture of tubercle bacilli’ [18].
Amongst his coworkers remained a centre of excellence and was the cradle for a new discipline: immunology. Amongst his coworkers were Emil von Behring and Paul Ehrlich, the founders of specific acquired immunity. At the same time, the Pasteur Institute, founded in 1888, served both as incentive and example for establishing Koch’s institute in Berlin in 1891.

Finale: the happy end

Although Koch's attempts to cure tuberculosis turned out exactly as possible without direct translation.

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Box 4. Koch’s school

Coworkers of Koch who became prominent and influential microbiologists:
- Friedrich Loeffler (1852–1915): discovery of the etiologic agent of diphtheria, Corynebacterium diphtheriae (1884); first description of a filterable ‘invisible’ pathogen (foot and mouth disease virus).
- Emil von Behring (1854–1917): passive vaccination against diphtheria and tetanus with antitoxins (Nobel Prize 1901).
- Shibasaburo Kitasato (1852–1931): passive vaccination against diphtheria with antitoxins (together with Emil von Behring), discovery of the etiologic agent of plague, Yersinia pestis (independently discovered by A.J.E. Yersin).
- Paul Ehrlich (1854–1915): concept of the side-chain theory in immunology; identification of complement; principles of chemotherapy; salvarsan (Nobel Prize 1908).

Figure 4. Robert Koch working in his laboratory in the Institute for Hygiene in Berlin, together with two of his staff scientists (between 1885 and 1900).