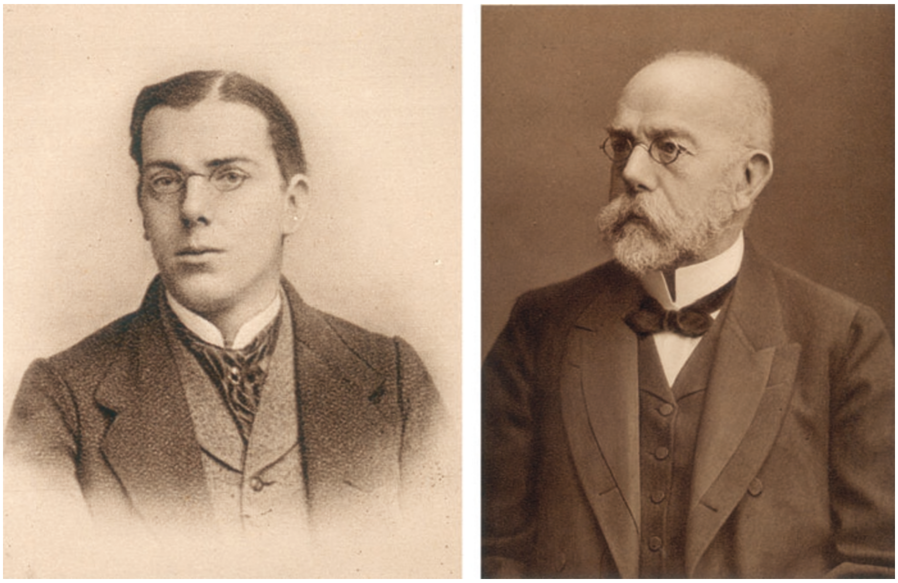
From bacteriology to immunology: the dualism of specificity

Stefan H E Kaufmann & Florian Winau

The 100th anniversary of Robert Koch's receiving the Nobel Prize reminds us of his achievements as a founder of modern bacteriology and as a forefather of immunology.

This year marks the 100th anniversary of Robert Koch's receiving the Nobel Prize for his groundbreaking work on tuberculosis. Koch's discovery of the etiological agent, *Mycobacterium tuberculosis*, was a landmark achievement in terms of both basic and applied research. Although he was a medical bacteriologist at heart, Koch's demonstration that distinct pathogens cause specific disease helped give birth to immunology as a discipline, at least at a theoretical level, because both subjects are intertwined by the dualism of specificity regarding pathogen assault and host defense. Different microorganisms induce unique pathologies based on the quality of the host reaction. Thus, pathogen specificity is also concomitantly the motif of specific immunity[1]. Here we will discuss the effect of Koch's bacteriological work on immunology.



Robert Koch, young and old. Left, Koch as student in Göttingen in 1864. Right, a portrait from around 1895 made by the Berlin court photographer Schaarschmidt. Provided by R. Winau of the Institute for History of Medicine (Charité, Berlin).

Founder of medical bacteriology

Although Robert Koch is best known for his discovery of the etiological agent of tuberculosis, he contributed far more to medical bacteriology. In 1876, he described *Bacillus anthracis* as the causative agent of anthrax and provided the first conclusive proof that a specific microbial agent causes a distinct type of infectious disease[2]. By fulfilling the criteria that define infectious disease etiology and were later called the 'Koch postulates'[3], he laid the basis for the new discipline of 'medical bacteriology'. Between 1880 and 1881, Koch decided to tackle what was at that time the main health problem of the Western world: tuberculosis. He described the results of his research first in an oral presentation given at the Berlin Physiological Society on 24 March 1882. The paper 'On the etiology of tuberculosis' was published on 10 April 1882, only three weeks later[4]. In that paper, Koch did not restrict his investigations to the pathogen but also considered the involvement of the host (**Fig. 1**). Although Koch would never lose his interest in tuberculosis, shortly after that discovery, a new challenge arose. Cholera spreading from its origins in India through Africa threatened Europe. By 1883, cholera had reached Egypt. A team led by Koch was sent to Alexandria, Egypt, where cholera rampaged, and they succeeded in isolating *Vibrio cholerae*[5,6]. Soon thereafter he successfully infected guinea pigs with *V. cholerae* after pretreatment with alkaline solution to neutralize the acidic gastric milieu[7].

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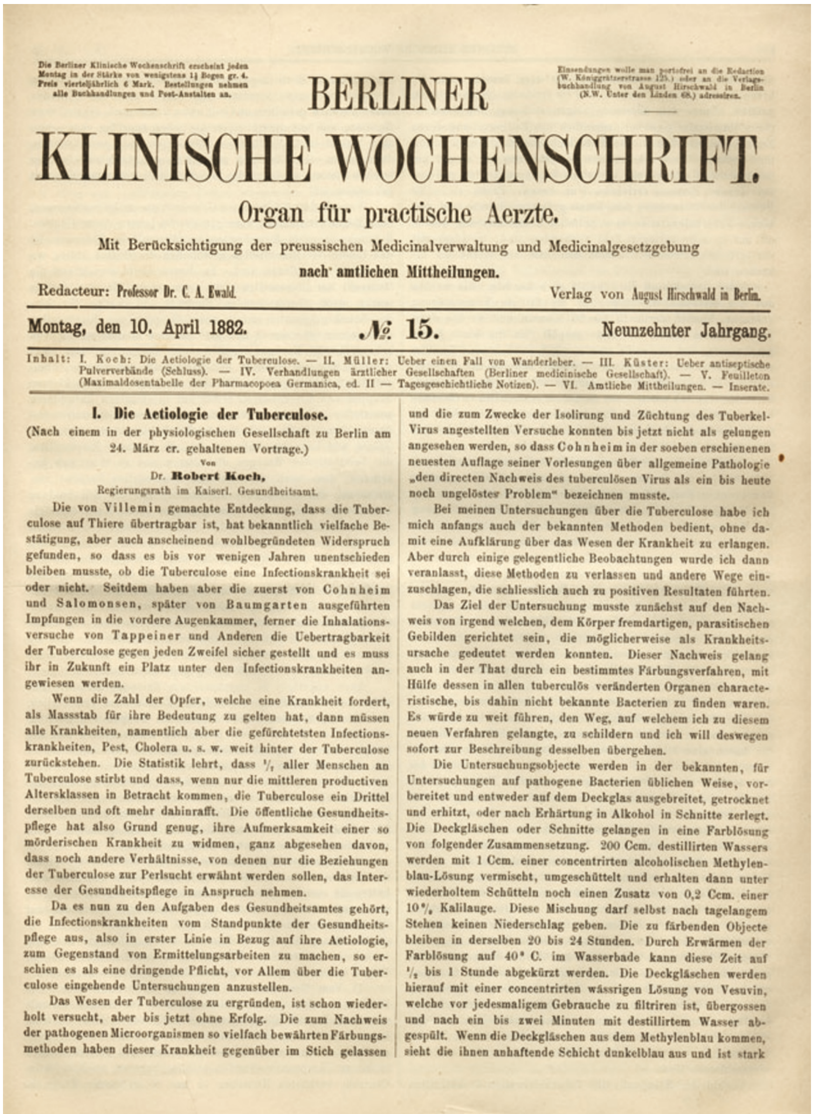
Mise en scène

What was new in Koch's approach? Louis Pasteur (1822–1895) had already provided strong evidence that life was not generated spontaneously and that microbial organisms were responsible for fermentation and



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I. Die Aetiologie der Tuberculose.

(Nach einem in der physiologischen Gesellschaft zu Berlin am 24. März cr. gehaltenen Vortrage.)

Von

Dr. **Robert Koch,**

Regierungsrath im Kaiserl. Gesundheitsamt.

Die von Villemin gemachte Entdeckung, dass die Tuberculose auf Thiere übertragbar ist, hat bekanntlich vielfache Bestätigung, aber auch anscheinend wohlbegründeten Widerspruch gefunden, so dass es bis vor wenigen Jahren unentschieden bleiben musste, ob die Tuberculose eine Infectionskrankheit sei oder nicht. Seitdem haben aber die zuerst von Cohnheim und Salomonsen, später von Baumgarten ausgeführten Impfungen in die vordere Augenkammer, ferner die Inhalationsversuche von Tappeiner und Anderen die Uebertragbarkeit der Tuberculose gegen jeden Zweifel sicher gestellt und es muss ihr in Zukunft ein Platz unter den Infectionskrankheiten angewiesen werden.

Wenn die Zahl der Opfer, welche eine Krankheit fordert, als Massstab für ihre Bedeutung zu gelten hat, dann müssen alle Krankheiten, namentlich aber die gefürchtetsten Infectionskrankheiten, Pest, Cholera u. s. w. weit hinter der Tuberculose zurückstehen. Die Statistik lehrt, dass $^1/_7$ aller Menschen an Tuberculose stirbt und dass, wenn nur die mittleren productiven Altersklassen in Betracht kommen, die Tuberculose ein Drittel derselben und oft mehr dahinrafft. Die öffentliche Gesundheitspflege hat also Grund genug, ihre Aufmerksamkeit einer so mörderischen Krankheit zu widmen, ganz abgesehen davon, dass noch andere Verhältnisse, von denen nur die Beziehungen der Tuberculose zur Perlsucht erwähnt werden sollen, das Interesse der Gesundheitspflege in Anspruch nehmen.

Da es nun zu den Aufgaben des Gesundheitsamtes gehört, die Infectionskrankheiten vom Standpunkte der Gesundheitspflege aus, also in erster Linie in Bezug auf ihre Aetiologie, zum Gegenstand von Ermittelungsarbeiten zu machen, so erschien es als eine dringende Pflicht, vor Allem über die Tuberculose eingehende Untersuchungen anzustellen.

Das Wesen der Tuberculose zu ergründen, ist schon wiederholt versucht, aber bis jetzt ohne Erfolg. Die zum Nachweis der pathogenen Microorganismen so vielfach bewährten Färbungsmethoden haben dieser Krankheit gegenüber im Stich gelassen und die zum Zwecke der Isolirung und Züchtung des Tuberkel-Virus angestellten Versuche konnten bis jetzt nicht als gelungen angesehen werden, so dass Cohnheim in der soeben erschienenen neuesten Auflage seiner Vorlesungen über allgemeine Pathologie „den directen Nachweis des tuberculösen Virus als ein bis heute noch ungelöster Problem" bezeichnen musste.

Bei meinen Untersuchungen über die Tuberculose habe ich mich anfangs auch der bekannten Methoden bedient, ohne damit eine Aufklärung über das Wesen der Krankheit zu erlangen. Aber durch einige gelegentliche Beobachtungen wurde ich dann veranlasst, diese Methoden zu verlassen und andere Wege einzuschlagen, die schliesslich auch zu positiven Resultaten führten.

Das Ziel der Untersuchung musste zunächst auf den Nachweis von irgend welchen, dem Körper fremdartigen, parasitischen Gebilden gerichtet sein, die möglicherweise als Krankheitsursache gedeutet werden konnten. Dieser Nachweis gelang auch in der That durch ein bestimmtes Färbungsverfahren, mit Hülfe dessen in allen tuberculös veränderten Organen characteristische, bis dahin nicht bekannte Bacterien zu finden waren. Es würde zu weit führen, den Weg, auf welchem ich zu diesem neuen Verfahren gelangte, zu schildern und ich will deswegen sofort zur Beschreibung desselben übergehen.

Die Untersuchungsobjecte werden in der bekannten, für Untersuchungen auf pathogene Bacterien üblichen Weise, vorbereitet und entweder auf dem Deckglas ausgebreitet, getrocknet und erhitzt, oder nach Erhärtung in Alkohol in Schnitte zerlegt. Die Deckgläschen oder Schnitte gelangen in eine Farblösung von folgender Zusammensetzung. 200 Ccm. destillirten Wassers werden mit 1 Ccm. einer concentrirten alcoholischen Methylenblau-Lösung vermischt, umgeschüttelt und erhalten dann unter wiederholtem Schütteln noch einen Zusatz von 0,2 Ccm. einer 10 % Kalilauge. Diese Mischung darf selbst nach tagelangem Stehen keinen Niederschlag geben. Die zu färbenden Objecte bleiben in derselben 20 bis 24 Stunden. Durch Erwärmen der Farblösung auf 40° C. im Wasserbade kann diese Zeit auf $^1/_2$ bis 1 Stunde abgekürzt werden. Die Deckgläschen werden hierauf mit einer concentrirten wässrigen Lösung von Vesuvin, welche vor jedesmaligem Gebrauche zu filtriren ist, übergossen und nach ein bis zwei Minuten mit destillirtem Wasser abgespült. Wenn die Deckgläschen aus dem Methylenblau kommen, sieht die ihnen anhaftende Schicht dunkelblau aus und ist stark

Robert Koch's seminal paper on tuberculosis. His talk 'On the etiology of tuberculosis' given at the Berlin Physiological Society on 24 March 1882 was published in the *Berliner Klinische Wochenschrift* (Berlin Clinical Weekly).

putrefaction[8,9]. Based on Pasteur's germ theory, the British surgeon Joseph Lister (1827–1912) assumed the microbial etiology of wound infections[10]. However, the idea prevalent at that time was that disease was transferred by 'miasma': fumes that were generated *de novo* during the process of degradation, fermentation and putrefaction. A second view opposing the germ theory of disease was promoted by Rudolf Virchow (1821–1902), a highly respected pathologist at the Charité in Berlin and founder of cellular pathology who claimed that all diseases originated from the body's cells themselves[11]. Although Virchow was skeptical about the mysterious miasma theories, he failed to realize that exogenous insults caused by a living contagion were the real cause. Although Virchow's strong influence in academia gave Koch a difficult time, he urged Koch to develop new methods to support his hypothesis and in doing so successfully established the new discipline of bacteriology.

The solitary researcher

How could a young country doctor succeed in founding a new discipline? Was he lucky enough to find a mentor who prepared a fertile soil and planted the seed from which the new idea could develop? Was he lucky enough to work at a vibrant center of excellence where new ideas were fostered and discussed by brilliant postdoctoral fellows? Was he lucky enough to find an institute where the appropriate infrastructure and technologies were waiting to be used for contesting his hypotheses experimentally? Of those prerequisites, none but perhaps the first was in part fulfilled. Koch studied in Göttingen, Germany, in 1862–1866, first mathematics and natural sciences and then medicine. There he was fortunate enough to attend the lectures of Jacob Henle (1809–1885), a pathologist and anatomist at the University of Göttingen, who had speculated that living contagions would be responsible for many diseases[12]. Henle probably implanted the idea of infectious diseases into Koch's scientific interests. Thereafter, however, he was left on his own. He became a country doctor, who performed his studies on anthrax in his own home in a part of his office that was separated by a curtain.

With no peer review and frequent flaws in research, Koch considered it critical that the quality of his first piece of work in a new discipline be approved by a rigorous peer. He approached the botanist Ferdinand Cohn (1828–1898) at the Institute for Plant Physiology at the University of Breslau (now Wroclaw, Poland). Koch not only showed his work to this highly respected scientist and his coworkers, he also convinced Cohn to make drawings of the microbes as he saw them under the microscope. Indeed, the original publication on anthrax includes figures drawn by Robert Koch and Ferdinand Cohn[2].

The effect of 'translational' medicine

The year 1890 marked the beginning of Robert Koch's attempts to prevent and treat tuberculosis. In a series of papers published between 1890 and 1891 (refs. 13,14), he described tuberculin and its application to diagnosis, but he also made the false claim that it was a remedy for the disease. Strong pressure from the government probably forced Koch to publicly announce a cure for tuberculosis at the Tenth International Congress of Medicine, which took place in Berlin in 1890 (ref. 15). At the end of his keynote lecture, Koch announced that he had discovered means for inhibiting the growth of tubercle bacilli in guinea pigs. Although he was very reluctant in his speech and clarified that he had only done preliminary animal experiments and no human studies, his announcement immediately excited the scientific community and soon thereafter electrified the public.

Between 1890 and 1891, Koch's remedy, tuberculin, was analyzed in a large multicentric clinical trial, as requested by the Ministry of Culture. The official report summarized the outcome of treatment of 1,769 patients[16]. Although safety studies and negative controls (untreated patients) were not included, the trial made it clear that tuberculin was almost completely ineffective.

In parallel, the medical research community in Berlin entered into intensive discussions about the value of tuberculin. The mostly supportive response, which prevailed until the end of 1890, was based on superficial clinical reports claiming cure of tuberculosis. By the beginning of 1891, the opinion gradually changed. When the first careful studies of autopsy material of patients who had died soon after treatment were discussed, the pathologist Rudolf Virchow emphasized the 'double-edged sword' character of tuberculin and reported that the destruction of the protective wall surrounding the tuberculous lesion in the affected organs would favor propagation of tubercle bacilli through the circulation and consequently exacerbate disease[17]. It is disappointing that Koch never publicly retracted his claim despite overwhelming evidence to the contrary. Yet with several subunit vaccines against tuberculosis entering clinical trials, perhaps Koch's approach was not completely wrong[18]. Tuberculin probably comprised abundant proteins that served as protective antigens and glycolipids, which acted as ligands for Toll-like receptors with potent adjuvanticity. Obviously, testing tuberculin as therapeutic remedy for patients with tuberculosis was the most straightforward approach. Its failure, however, may have prevented its subsequent testing as a pre-exposure vaccine, which might have been partially successful.

The Koch school

Building schools represents an essential part of transmitting knowledge and ideas to succeeding generations. Robert Koch was an excellent teacher, and this is reflected in how he organized his laboratory. He started out investigating microorganisms because as district general practitioner he was often confronted with patients suffering from medical conditions of unknown etiology, which today are called infectious diseases. Throughout his professional life, dissecting the mechanisms underlying infectious disease remained the driving force of his research. Although he had started as a complete unknown without support from the outside and was never fully integrated into university life, he successfully founded a school of excellent researchers. Originally, Koch created a laboratory with 'soft' hierarchy levels, thus preventing the separation of ideas from experimental verification. He directly imparted his knowledge to his coworkers when he built up his small research group at the Imperial Health Office (Berlin) between 1880 and 1885, where he had been granted positions for two staff scientists. Typically, Koch was found at the bench in the middle of his laboratory surrounded by his younger coworkers. Koch maintained the structural organization during the expansion of his laboratory at the Institute of Hygiene (Berlin) between 1885 and 1891. As director, he remained the principal investigator, and the exchange of new ideas among colleagues was fostered without impediment. Although Koch disliked his affiliation with the university and his academic duties such as student lecturing, it was during this period at the Imperial Health Office and the Institute of Hygiene that Koch built his scientific school.

Figure 1 Granulomatous lesion from tuberculosis specimen. Faint blue rods represent *Mycobacterium tuberculosis* bordered by cells (in red) called 'macrophage-derived epitheloid cells'. Drawing from Reports of the Imperial Health Office (Berlin, 1884; Table 5).

The first generation of Koch's coworkers focused on medical bacteriology taking advantage of methods and ideas developed by their mentor. Some of them became famous scientists in their own right, such as Georg Gaffky (1850–1918), a codiscoverer of the cholera bacillus[19], and Friedrich Loeffler (1852–1915), discoverer of the etiologic agent of diphtheria, *Corynebacterium diphtheriae*[20], and of the first filterable pathogen (virus), the agent of foot-and-mouth disease.

The next generation turned their interest toward the host response and became cofounders of immunology as a new discipline. Of note are Emil von Behring[21] (1854–1917), the discoverer of passive vaccination against diphtheria and tetanus (a Nobel laureate in 1901), and Paul Ehrlich[22] (1854–1915), who developed the side-chain theory and the principles of chemotherapy (a Nobel laureate in 1908). With the increasing reputation of Koch's institute, many foreign researchers joined him, most notably Shibasaburo Kitasato (1852–1931), coauthor with Emil von Behring on the work on immunity to diphtheria and tetanus[23].

Emil von Behring started his career with Robert Koch in 1889. The production of antisera against diphtheria required considerable funding, provided by the Farbwerke Hoechst, which granted the license for producing these biological agents. Based on Behring's discovery and Ehrlich's technical expertise in standardizing antibody activity, a contract between Ehrlich and Behring to produce a highly effective antiserum against diphtheria was signed. Of the two, Ehrlich was probably the more influential mentor for the newly emerging field of immunology. Ehrlich joined Koch in 1890 first as a medical doctor overlooking clinical trials with tuberculin and later as an independent researcher in the newly founded Institute for Infectious Diseases (Berlin).

Koch's school was initially highly successful due to its size and structural organization. However, Koch clearly did not understand the secrets of his success, as illustrated by his decision to found a new Institute for Infectious Diseases (Berlin). The new institute, inaugurated in 1891, was characterized by a colossal building, independence from the university and close association with the government, division into different departments and a highly diverse program of research. Soon after its inauguration, the highly intertwined network of coworkers broke down. Gaffky and Loeffler became directors of their own Institutes of Hygiene, Emil von Behring moved to Marburg, Paul Ehrlich moved to Frankfurt and Kitasato moved back to Japan. Communication among the different departments vanished and because of the rigid structure, the hierarchical levels of the institute gradually became isolated. It remains unclear whether Koch's lengthy expeditions to the tropics during his time at the Institute for Infectious Diseases were the reason for or the result of its divergent activities. Of course, the Institute for Infectious Diseases still produced important work on medical bacteriology and immunology. However, this work was less the result of the Koch school in a narrow sense but instead reflected individual achievements. Paul Ehrlich perhaps represents the best example of this, as he was initially associated with the institute but did mostly autonomous research.

The French connection

Louis Pasteur, who created and promoted the germ theory, was a chemist by education with an interest in application, including vaccine development. In 1879, 1881 and 1885, he described successful vaccination against fowl cholera in chicken, against anthrax in sheep and against rabies in humans. The success of these broadly publicized accomplishments led to the foundation of the Pasteur Institute

(Paris) in 1886 and its opening in 1888. From its beginning, this institute was a private foundation with only partial support from the government. The Pasteur Institute rapidly developed into a successful center of excellence with high public regard, not least because of Pasteur's unique ability to combine top science with public awareness. Probably this success story served as a strong incentive for the foundation of the Institute for Infectious Diseases in Berlin[24].

The newly emerging scientific disciplines of medical bacteriology and immunology in the second half of the nineteenth century were advanced mainly by these two European centers built around Louis Pasteur in Paris and Robert Koch in Berlin[25]. The relationship between the French and German schools has often been characterized by animosity and considerable antagonism of 'cellularists' and 'humoralists'[26]. Neither label is correct. Emil von Behring and Paul Ehrlich invented serum therapy and were therefore interested mainly in the humoral immune response, whereas Elie Metchnikov (1845–1916) at the Pasteur Institute promoted cellular immunity based on his investigation on phagocytosis[27]. However, research directions on both sides were far more intertwined. Thus, humoral immunity at the Pasteur Institute was studied by Emile Roux (1853–1933), who discovered the diphtheria toxin, and by Jules Bordet (1870–1961), who discovered the complement system. Yet both were also close collaborators of Metchnikov[28,29]. Similarly, Ehrlich never neglected the importance of cellular immunity. Both Behring and Roux were honored by the Académie des Sciences of France for their work on passive vaccination against diphtheria. It is true that Behring had a challenging character; however, he quarreled not only with French scientists but also with his former mentor Koch and with his long-time colleague Ehrlich and did not select his opponents on a national basis. Thus, personal relations between Paris and Berlin were surprisingly amicable, as highlighted by the fact that Roux and Metchnikov were godfathers of two of Emil von Behring's children.

Major conflicts, however, existed between Pasteur and Koch; these were not due to private or national animosity but instead to different cultural backgrounds and contrasting concepts of bacteriology. Pasteur was strongly influenced by the materialistic philosophy of nineteenth century France and hence focused on pragmatic research with a direct link to application. He became increasingly interested in vaccination based on the principle of pathogen attenuation. Hence, he can be considered as direct successor of Edward Jenner's pragmatic approach to infectious disease control.

In contrast, Koch grew up in an environment that had just shifted from pure idealism toward new realism. This process was facilitated by the revolution of 1848 in Germany and was paralleled by a profound industrialization process. Yet modern nineteenth century science in Germany still retained a large part of the idealistic 'l'art pour l'art' attitude. Koch was interested in understanding human disease but mainly focused his investigations on deciphering the basic rules for a unifying theory of medical bacteriology. Koch described the etiology of anthrax, whereas Pasteur attenuated anthrax bacteria for vaccination purposes. The dispute between the two scientists centered on anthrax[30], because Koch realized that the limiting dilution cultures used by Pasteur were insufficient to yield pure bacterial cultures. The effects of vaccination against a defined bacterial population required the culture of single colonies on solid medium. This was developed by Koch and was later adopted by scientists at the Pasteur Institute. However, Pasteur's vaccination against anthrax was extraordinarily successful, and Koch's stubbornness prevented reconciliation. Pasteur's emphasis on the applied aspects of vaccination and Koch's elucidation of the general principles of medical bacteriology render them complementary contributors to this field[31].

Concluding remarks

Robert Koch began as a scientist interested in identifying the causative agents of infectious diseases, but he was more than a 'germ hunter'. He developed the methods and formulated the general principles that established modern bacteriology as a verifiable scientific discipline. The specificity of pathogens became a principal hallmark of his way of scientific thinking. Because of the dualism of specificity of pathogen and host response, the development of modern bacteriology gave rise to the birth of immunology. Thus, Robert Koch paved the way for immunology put forward by his disciple Paul Ehrlich.

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1. Mazumdar, P.M. *Species and Specificity: an Interpretation of the History of Immunology* (Cambridge University Press, Cambridge, UK, 1995).
2. Koch, R. *Beitr. z. Biol. d. Pflanzen* **2**, 277–310 (1876).
3. Carter, K.C. *Med. Hist.* **29**, 353–374 (1985).
4. Koch, R. *Berliner klin. Wochenschr.* **19**, 221–230 (1882).
5. Koch, R. *Dt. med. Wochenschr.* **9**, 743–744 (1883).
6. Ogawa, M. *Bull. Hist. Med.* **74**, 671–707 (2000).
7. Koch, R. *Dt. med. Wochenschr.* **10**, 725–728 (1884).
8. Pasteur, L. *Ann. d. Sc. naturelles* **44**, 5–98 (1860).
9. Silverstein, A.M. *Hist. Philos. Life Sci.* **22**, 29–41 (2000).
10. Lister, J. *Verhandlung des X. Internationalen Medicinischen Congresses* Vol 1 (ed. Redactionscomité) 1–351 (August Hirschwald, Berlin, 1891).
11. Virchow, R. *Die Zellularpathologie* Vol 1, 1–582 (August Hirschwald, Berlin, 1871).
12. Henle, J. *Von den Miasmen und Kontagien und von den miasmatisch-kontagiösen Krankheiten* (ed. Sudhoff, K.) 12–88 (Johann Ambrosius Barth, Leipzig, 1910).
13. Koch, R. *Dt. med. Wochenschr.* **16**, 1029–1032 (1890).
14. Koch, R. *Dt. med. Wochenschr.* **38**, 57–58 (1891).
15. Koch, R. *Hirschwald* **1**, 35–47 (1891).
16. Guttstadt, A. *Die Wirksamkeit des Kochschen Heilmittels gegen Tuberculose. Amtliche Berichte der Kliniken, Polykliniken und Pathologisch/Anatomischen Institute der Preußischen Universitäten* (ed. Guttstadt, A.) 1–905 (Julius Springer, Berlin, 1891).
17. Virchow, R. *Dt. med. Wochenschr.* **17**, 131–134 (1891).
18. Kaufmann, S.H. & McMichael, A.J. *Nat. Med.* **11**, S33–S44 (2005).
19. Gaffky, G. Arbeiten a. d. Kaiserl. Gesundheitsamte **3**, 1–272 (1887).
20. Loeffler, F. *Mitth. a. d. Kaiserl. Gesundheitsamtes* **2**, 421–499 (1884).
21. Winau, F. & Winau, R. *Microbes Infect.* **4**, 185–188 (2002).
22. Winau, F., Westphal, O. & Winau, R. *Microbes Infect.* **6**, 786–789 (2004).
23. Behring, E. & Kitasato, S. *Dt. med. Wochenschr.* **16**, 1113–1114 (1890).
24. Weindling, P. in *The Laboratory Revolution in Medicine* (eds. Cunningham, A. & Williams, P.) 170–188 (Cambridge University Press, Cambridge, UK, 1989).
25. Mendelsohn, J.A. *Hist. Philos. Life Sci.* **24**, 3–36 (2002).
26. Silverstein, A.M. *Nat. Immunol.* **4**, 425–428 (2003).
27. Chernyak, L. & Tauber, A.I. *Cell. Immunol.* **117**, 218–233 (1988).
28. Tauber, A.I. & Chernyak, L. *Cell. Immunol.* **121**, 447–473 (1989).
29. Tauber, A.I. *Cell. Immunol.* **139**, 505–530 (1992).
30. Carter, K.C. *Bull. Hist. Med.* **62**, 42–57 (1988).
31. Heymann, B. *Robert Koch* Vol 1 (ed. Ostwald, W.) (Akademische Verlagsgesellschaft, Leipzig, 1932).