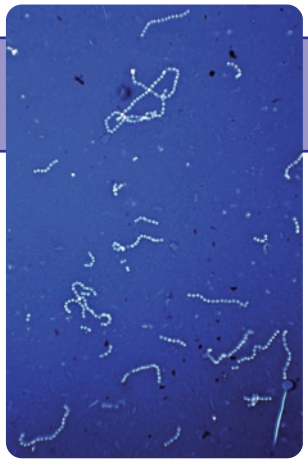

Chapter 1

Microbiology, microbial pathogens and infectious disease

For much of human history, the existence of microorganisms was unknown. Disease was often attributed to evil forces associated with disturbances in the upper atmosphere, poisonous vapours called miasmas, supernatural events and other influences unrelated to biology. Awareness of the possible existence of forms of life not visible to the naked eye emerged slowly. As early as 1546, in his treatise *De Contagione*, Girolamo Fracastoro suggested that animate agents were responsible for disease. The development of the microscope around 1600 offered a means of exploring minute living entities, and the amateur Dutch scientist, Antonie van Leeuwenhoek, took a keen interest in the examination of water, fluids and organic material. In fluids, he observed large numbers of motile structures, not visible to the naked eye, which he called 'animalcules'. In 1675, van Leeuwenhoek recorded the structures he observed, which were probably bacteria, yeasts and protozoa.

During the past 150 years, major developments have taken place in microbiological concepts, techniques and applications. The study of microorganisms began with theories relating to the causes of infectious diseases and continued with the development of microscopy, which confirmed the existence of microorganisms visible only by substantial magnification. Modern microbiology encompasses the study of bacteria, fungi, viruses, and other microscopic and submicroscopic organisms (Fig. 1.1). Concepts of infectious diseases have been closely related to the demonstration of organisms too small to be observed without magnification and to the isolation and characterization of these small organisms, termed microorganisms. In veterinary microbiology, emphasis is placed on those microorganisms associated with infectious diseases of animals. Progressive developments have contributed to the rapid expansion of our knowledge of the microbial world and the establishment of microbiology as a subject of major importance not only in human and animal health but also in food processing and preservation.

The earliest forms of life on this planet are presumed to have had characteristics resembling those of bacteria, most likely anaerobic bacteria. It is postulated that prokaryotes evolved from primitive forms of life and that the subsequent availability of oxygen resulting from photosynthesis contributed to microbial diversity. A biological classification system encompassing all organisms in the tree of life and known as the two-domain system has been proposed. This model challenges the widely accepted three-domain system of Bacteria, Archaea and Eukarya. It places all organisms in two domains, Bacteria and Archaea. Although Bacteria and Archaea exchange DNA, the two domains of prokaryotes are very distinct; for example, archaea have membranes containing ether lipids, whereas bacteria have membranes containing ester lipids. Genetic and proteomic studies support the suggestion that eukaryotes originated within the Archaea domain from a group of archaea known as Asgard archaea. A further twist to the evolution of eukaryotes is the fact that organelles, such as mitochondria and chloroplasts, evolved from free-living alpha-proteobacteria and cyanobacteria, respectively, and were acquired

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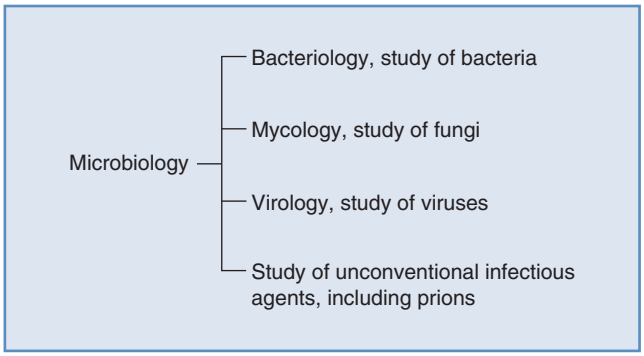

Figure 1.1 Subdivisions of microbiology, a subject which has areas of common interest with pathology, immunology, pharmacology, medicine and therapeutics.

by a proto-eukaryote in a process termed endosymbiosis. The approximate chronological sequence of evolutionary events relating to the emergence of microbial life and, subsequently, eukaryotic cells is outlined in Fig. 1.2. This proposed scheme is based on limited factual information, some deriving from information gleaned from fossilized remains of prokaryotic cells approximately 3.5 billion years old and also from studies of ribosomal RNA among microorganisms.

Once the existence of the microbial world was accepted, two questions came to dominate the debates of scientists of the nineteenth century. The first question concerned 'spontaneous generation' as an explanation for the emergence of life from decaying organic matter. This was a commonly held view for many centuries, strongly influenced by the writings of classical Greek and Roman scholars, many of whom espoused the view of spontaneous generation of small living entities. It was postulated that life began as a consequence of putrefaction or some other associated change in organic matter. The occurrence of maggots on putrefying meat was taken as evidence of spontaneous generation. A number of practical experiments aimed at testing this concept were carried out, often with equivocal results. The Italian physician and naturalist Francesco Reddi (1626–1697) demonstrated that maggots developed in meat only when flies laid their eggs on it. In the mid-eighteenth century, the English naturalist John Needham investigated the effect of boiling broth on the survival of microorganisms. He claimed to have detected microorganisms in boiled broth several days later. Needham's experimental procedures were subsequently shown to have been unreliable. In 1769, Lazzaro Spallanzani repeated Needham's experiments and demonstrated that no organisms survived in broth boiled for 1 hour. Needham argued that air was essential for all life and that Spallanzani had excluded air from the flasks containing broth. As a defined branch of science, microbiology could not advance until the concept of spontaneous generation

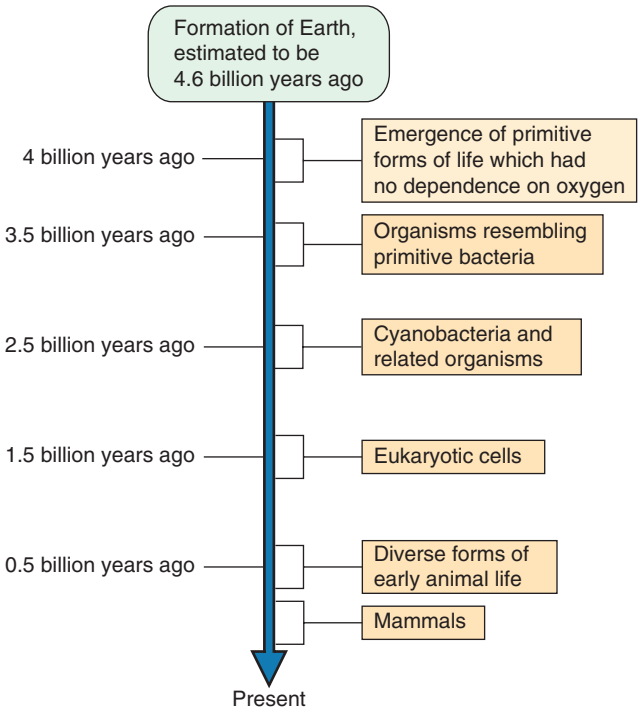

Figure 1.2 Chronological sequence of biological events from the formation of Earth, relating to the evolution of different forms of microbial life and, later, eukaryotic cells. Although supporting scientific evidence documenting the earliest forms of microbial life is not currently available, data from microfossils confirm the existence of organisms resembling cyanobacteria approximately 3.5 billion years ago.

was disproved. When the French chemist Louis Pasteur (1822–1895) became involved in investigations relating to microbiology, his careful planning and intuitive understanding of biology brought a new energy and appropriate methodology, which conclusively refuted the prevailing theories of spontaneous generation. Using a specially designed 'swan-neck' flask that allowed air to enter but excluded airborne bacteria, he was able to show that water, sterilized by boiling, remained free of bacteria. Furthermore, he demonstrated that contamination of the nutrient broth when exposed to air resulted from microorganisms in dust particles settling on the fluid.

Pasteur's interest in spontaneous generation had been prompted by experiments that he had conducted on spoilage during the fermentation of beet alcohol. He showed that a contaminating yeast that produced lactic acid during fermentation, and which differed morphologically from brewers' yeast, was responsible for the spoilage. He deduced that both alcoholic and lactic fermentation resulted from metabolism and replication of the living yeast cells. The solution to the spoilage problem during fermentation of wine and beer products lay in heating the raw materials to about 120°F (49°C) in order to kill contaminating

microorganisms prior to the addition of the appropriate yeast cells. This process, now known as pasteurization, is widely used to reduce microbial contamination and prolong the shelf life of milk and some other foods.

The second question concerned the role of microbes in infectious diseases and whether particular microbes caused specific diseases. Since the time of Hippocrates in the fifth century B.C., contagious disease had been ascribed to miasmas (poisonous vapours). Towards the middle of the nineteenth century, the pioneering work of Louis Pasteur and Robert Koch (1834–1910) confirmed the microbial aetiology of infectious diseases. An important technical advance, which stemmed from Pasteur's fermentation studies, was the development of a fluid medium suitable for culturing yeast cells. He then developed other liquid media containing specific ingredients that favoured the growth of particular pathogenic bacteria. It was this development which eventually allowed him to formulate the germ theory of disease. This theory formed the basis for Pasteur's experiments on vaccination against fowl cholera, anthrax and rabies. An additional practical application of the theory was the introduction of phenol as a disinfectant for surgical procedures by the British surgeon Joseph Lister.

Having observed bacilli in the blood of animals that had died from anthrax, Koch demonstrated their pathogenicity by injecting mice with the blood. The injected mice died, and the bacilli were present in preparations from their swollen spleens. He was also able to transfer the infection from mouse to mouse and to demonstrate the bacilli in each newly infected mouse. Initially, Koch used blood serum for growing the anthrax bacillus *in vitro*. Later, he developed solid media, which allowed the isolation of individual bacterial colonies. Using a solid medium, he was eventually able to isolate *Mycobacterium tuberculosis* from the tissues of an experimental animal in which he had demonstrated microscopically the presence of the organism. As a result of these observations, Koch formulated certain principles for proving that a specific microorganism caused a particular disease (Box 1.1).

Box 1.1 Koch's postulates.

- The pathogenic microorganism must be present in every case of the disease but absent from healthy animals
- The suspected microorganism must be isolated and grown in pure culture
- The same disease must occur when the isolated microorganism is injected into healthy susceptible animals
- The same microorganisms must be isolated again from the injected animals that developed disease

Pasteur's germ theory of disease and Koch's postulates are the two cornerstones on which microbiology is based, and without which this branch of biology could not have advanced.

By the end of the nineteenth century, a number of important infectious diseases had been confirmed as bacterial in origin. Both Pasteur and Koch contributed to the identification and confirmation of the causal agent of anthrax. Pasteur demonstrated that fowl cholera, malignant oedema and suppurative lesions were each associated with a specific bacterial infection. The causative organisms of tuberculosis and typhoid fever were recognized by Koch and his associates. Other bacterial agents responsible for serious infectious diseases, including glanders, gas gangrene, diphtheria and dysentery, were isolated by laboratory scientists in Europe, North America and Japan.

However, the basic technical approaches, pioneered by Pasteur and Koch, failed to shed light on the causes of many other serious infectious diseases, such as rabies, smallpox, foot-and-mouth disease, and rinderpest. Despite the absence of specific knowledge about the aetiology of these diseases, successful vaccines were introduced both for smallpox, by Edward Jenner in the late eighteenth century, and for rabies, by Pasteur and his associates in the latter half of the nineteenth century. The development by Pasteur's co-worker, Charles Chamberland, of the porcelain philtre to produce bacteriologically sterile water for use in culture media eventually facilitated the isolation of the filterable agents that caused viral diseases. Remarkably, the technique was first used to elucidate the cause of a plant viral disease, tobacco mosaic disease. The term virus comes from the Latin word for poison.

Dmitri Ivanovsky, a Russian scientist, reported in 1892 that it was possible to transmit tobacco mosaic disease from diseased to healthy plants using filtered leaf extract as inoculum. The filters used by Ivanovsky were Chamberland porcelain filters designed to remove bacteria from drinking water. In 1898, Martinus Beijerinck, unaware of the work of Ivanovsky, also demonstrated the filterability of the agent of tobacco mosaic disease. Moreover, he realized that the disease could not be due to a toxin, as the filtered sap from infected plants could be used for serial transmission of the disease without loss of potency. In the same year, Loeffler and Frosch identified the first filterable agent from animals, the virus of foot-and-mouth disease. Yellow fever virus, a filterable agent pathogenic for humans, was described by Walter Reed and his team in 1901. Ellerman and Bang, in 1908, demonstrated the oncogenic potential of a filterable agent, the cause of avian leukosis. In 1915, Frederick Twort observed that bacteria were susceptible to a filterable agent, and

2 years later, Felix d'Herelle made a similar observation. D'Herelle named these viruses 'bacteriophages' and developed a technique for establishing their concentration in active preparations. Bacteriophages have proved to be particularly useful in studies on viral replication and bacterial genetics.

Initially, the only method available for recovering large quantities of the virus was through infecting susceptible animals. In 1913, Steinhardt and his colleagues succeeded in growing vaccinia virus in explants of guinea pig cornea embedded in clotted plasma. Some 20 years later, Furth and Sturmia used mice as a host species for propagating viruses, while Woodruff and Goodpasture were successful in propagating fowlpox virus on the chorioallantoic membrane of embryonated eggs. A major advance was made in the early 1950s with the development of single-cell cultures. Factors critical in this development included the availability of antibiotics to control bacterial contamination and the use of trypsin to obtain cell suspensions from embryonic or adult tissue. The separated cells could then be grown as monolayers on glass surfaces. Continuous cell lines, capable of multiplying indefinitely, provided a reliable source of cells for virus cultivation.

In 1887, Buist observed vaccinia virus using a light microscope. However, because of the limited resolving power of this type of microscopy, the structure of the virus was not discernible. In 1939, Kausche and his co-workers employed the newly developed electron microscope and a metal shadowing technique to identify tobacco mosaic virus (TMV) in purified preparations. Ultrastructural studies of viruses were greatly expanded and enhanced in the 1950s by the development of negative staining and methods for cutting ultrathin sections. X-ray diffraction methods have been applied to viruses since the 1930s, when it was discovered that simple viruses could be crystallized. The first complete high-resolution structure of a crystalline virus, tomato bushy stunt virus, was obtained by Harrison and his co-workers in 1978. Computer analysis of the diffraction patterns obtained by such studies has contributed to knowledge of the molecular structure of viruses.

The crystallization of TMV by Stanley in 1935 provided a boost to the analysis of the chemical composition of viruses. In 1937, Bawden and Pirie showed that TMV contained nucleic acid as well as proteins and helped to promote the idea that viruses consisted of nucleic acid contained within a protein coat. Having elucidated the structure of DNA and observed the limited coding capacity of viral nucleic acid, Watson and Crick in 1956 suggested that viral nucleic acid was surrounded by a shell of identical protein subunits. In 1962, Lwoff and his colleagues proposed a universal system on which the modern classification of viruses is based. The method of classification proposed was based on the following criteria: (1) the type of nucleic acid; (2) the symmetry of the virus; (3) the presence or absence of an envelope; and (4) the diameter of the nucleocapsid (helical viruses) or the number of capsomers (icosahedral viruses). The discovery of the enzyme reverse transcriptase in 1970 by Temin and Baltimore helped to elucidate retrovirus replication and provided an essential tool for producing complementary DNA (cDNA). This ushered in the recombinant DNA revolution. The study of retroviruses has made a substantial contribution to the advancement of basic research in neoplasia and the role of oncogenes in the emergence of malignant tumours.

As knowledge of disease processes has increased over time, it has become clear for certain infectious diseases that Koch's postulates could not be fulfilled and that no single set of criteria is likely to provide proof of causation in all cases. For example, for certain diseases, such as feline infectious peritonitis, it is possible to isolate feline coronavirus from both healthy individuals as well as from cats showing signs of the disease. In other cases, certain viruses, such as poxviruses or papillomaviruses, proved difficult if not impossible to isolate and grow in pure culture. In many cases, these viruses were eventually isolated after many years of effort, but to this day, the poxvirus that causes molluscum contagiosum has never been grown in tissue culture. However, guidelines are valuable in weighing up the evidence for causation, and various revisions have been proposed. During his President's address to the Royal Society of Medicine on the subject of environmental and occupational medicine, Sir Austin Bradford Hill proposed nine epidemiological criteria in evaluating a causal relationship (Hill, 1965). Population-level datasets of serological reactivity against specific viral antigens were used to demonstrate a causal relationship between Epstein–Barr virus and infectious mononucleosis, prompting Alfred Evans to propose his 'Elements of Immunological Proof of Causation' and his unified concept of causation (Evans, 1976). The increasing use of molecular techniques to detect and identify infectious agents prompted a further revision of Koch's postulates by Stanley Falkow in 1988, termed 'molecular Koch's postulates'. The three postulates (Falkow, 2004) are:

1. The phenotype or property under investigation should be associated with pathogenic members of a genus or pathogenic strains of a species.
2. Specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence.
3. Reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity.

However, the discussion on causation continues apace. In 1996, Fredricks and Relman reviewed previous guidelines and proposed seven molecular guidelines for establishing the causation of microbial disease.

The future of microbiology is bright. Advances in DNA sequencing technology have resulted in faster throughput and lower costs to such an extent that we can now investigate the gut microbiome or the amazing complexity of the environmental microbiome without ever having to isolate and culture the myriad of microbial species involved. It has also become clear that the One Health approach, encompassing humans, animals and the environment, is a concept of fundamental importance in tackling global issues, such as antimicrobial resistance, emerging diseases and climate change. Curiosity-led scientific inquiry has delivered extraordinary results and the tools to reshape our world. The identification of a sophisticated prokaryotic antiviral defence system, the CRISPR-Cas9 enzyme system, has opened up a whole range of new applications from biotechnology to human gene therapy (Doudna and Charpentier, 2014). As our understanding expands, so too does the responsibility of ensuring that the knowledge is put to good use.

References

Doudna, J. and Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346, 1258096. http://dx.doi.org/10.1126/science.1258096

Evans, A.S. (1976). Causation and disease: the Henle-Koch postulates re-visited. *Yale Journal of Biology and Medicine*, 49, 175–195.

Falkow, S. (2004). Molecular Koch's postulates applied to bacterial pathogenicity – a personal recollection 15 years later. *Nature Reviews Microbiology*, 2, 67–72. http://dx.doi.org/10.1038/nrmicro799

Fredricks, D. and Relman, D. (1996). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, 9, 18–33. http://dx.doi.org/10.1128/CMR.9.1.18

Hill, A.B. (1965). The environment and disease: association or causation? *Proceedings of the Royal Society of Medicine*, 58, 295–300. http://dx.doi.org/10.1177/003591576505800503

Further reading

Dunlop, R.H. and Williams, D.J. (1996). *Veterinary Medicine: An Illustrated History*. Mosby, St. Louis, Missouri.

Frankland, P. and Frankland, P. (1901). *Pasteur*. Cassell and Company, London.

Lechevalier, H.A. and Solotorovsky, M. (1965). *Three Centuries of Microbiology*. McGraw-Hill, New York.

Pelczar Jr., M.J., Chan, E.C.S. and Krieg, N.R. (1993). *Microbiology Concepts and Applications*. McGraw-Hill, New York.

Porter, R. (1999). *The Greatest Benefit to Mankind: A Medical History of Humanity*. Fontana Press, London.

Prescott, L.M., Harley, J.P. and Klein, D.A. (2002). *Microbiology*. Fifth Edition. McGraw-Hill, New York.

van Doorn, J., Luirink, J., van der Oost, J., *et al.* (2017). *Mighty Microbes: The Amazing World of Microorganisms*. Microcanon Foundation, The Netherlands.

van Regenmortel, M.H.V. (1990). Virus species, a much overlooked but essential concept in virus classification. *Intervirology*, 31, 241–254. http://dx.doi.org/10.1159/000150159