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8.1 Pathogenic microorganisms and the host CONTENTS 8.1.1 Biology of pathogenic microorganisms 651 Duncan J. Maskell and James L.N. Wood 8.1.2 Clinical features and general management of patients with severe infections 656 Peter Watkinson and Duncan Young 8.1.1 Biology of pathogenic microorganisms Duncan J. Maskell and James L.N. Wood ESSENTIALS Microorganisms are present at most imaginable sites on the planet, and have evolved to occupy these ecological niches successfully. A host animal is simply another ecological niche to be occupied. The ability to cause disease may in some cases be an accidental bystander event, or it may be the result of evolutionary processes that have led to specific mechanisms allowing the pathogen to exploit the rich source of nutrients present in the host, and then be transmitted to another fresh host. Pathogenicity often relies on a series of steps, with specific and often distinct mechanisms operating at each of them. Some types of pathogen must adapt to the host environment by altering gene expression, and most must retain the ability to be transmitted readily between hosts. Specific mechanisms have evolved in microorganisms for the exploitation of the host and for evasion or avoidance of the innate and acquired immune systems. The advent and application of hyper-rapid and ultra-high throughput whole genome scale sequencing technologies is providing a mass of information, which has started to change fundamentally our way of looking at infectious diseases and our understanding of how pathogens work. This should enable the development of new intervention strategies, especially vaccines and antimicrobials, but the complexity of some of the biological mechanisms involved may make this a difficult exercise. Furthermore, pathogens can vary and evolve rapidly, and thus are likely to remain one step ahead of these strategies. We live in a rapidly changing world. New pathogens will emerge to exploit new circumstances presented by changes in society, and ancient scourges will remain and re-emerge to plague us. Many of the new infectious disease challenges will arise from animals, and will be zoonoses, at least in the early stages of their emergence. It is therefore probably more important in this field than in any other to develop the vision of 'One Medicine', with medical and veterinary clinicians and basic scientists working together, if we are to give ourselves the best chance of success in warding off threats from global infectious diseases. Introduction Microorganisms occupy almost all imaginable ecological niches. Microbes have been isolated from deep-sea sites, where

they survive very high pressures, from extremely cold and extremely hot regions, where hyperthermophiles grow optimally at temperatures well in excess of 100°C, and even from rocks, where they can exploit chemical substrates for energy generation. It is no wonder, then, that microorganisms should also exploit other living organisms as potential habitats, from viruses that use other microorganisms as hosts (e.g. bacteriophage) through to microorganisms that occupy various ecological niches within and upon the mammalian body, sometimes to the benefit and sometimes to the severe detriment of the host. Microorganisms have been supremely successful in evolutionary terms and they contain enormous untapped reserves of biodiversity, much of which is to be found in those that we can neither isolate nor grow and which make up most microbes on the planet. Since microorganisms reproduce much more rapidly than their mammalian hosts and have several specialized mechanisms for horizontal gene transfer, it is not surprising that they are often able to evolve quickly to stay one step ahead of any mechanisms that exist, or are invented by humans, to control them. The control of infectious diseases over the last century or so has been a major achievement, relying mainly on improved public health systems and social conditions, as well as on technological advances such as antimicrobial drugs and vaccines. Optimism about winning the battle with infectious diseases was bolstered by the eradication of smallpox in 1977, achieved by a monumental worldwide public health and vaccination programme. But, as Aldous Huxley

652 SECTION 8 Infectious diseases wrote, 'Hubris against the . . . order of Nature would be followed by its appropriate Nemesis', and so it was that very soon afterwards we had to learn to cope with the global catastrophe that is AIDS, along with the resurgence of ancient killers such as tuberculosis, and the emergence of apparently new or re-emergent threats such as bovine spongiform encephalopathy (BSE) and West Nile fever. In 2009, the world found itself dealing with the long-predicted global pandemic of influenza which severely stretched and tested the ingenuity and organizational abilities of international human society in its attempts to control the impact of the virus. That this pandemic had a relatively minor impact, despite rapid and widespread dissemination and transmission, was largely due to the generally mild nature of disease caused by this virus. Lessons for public health regarding control are still being learned from this incident. Far more devastating, at least locally, has been the Ebola epidemic in three countries in West Africa between 2014 and 2016, a catastrophe that has had far reaching consequences on health service infrastructures in those countries and on international responses to epidemic diseases. New disease threats will continue to emerge, leading to consequences that are difficult to predict, such as the emergence of Zika virus as a recent example. Pathogenicity in stages It is important to break down pathogenesis into different steps and stages. Most viral and bacterial pathogens enter the host via the mucosa of the respiratory, gastrointestinal, or genitourinary tracts, although some important pathogens are introduced by injection from insect vectors or through abraded or wounded skin. Most pathogens then must stick to a surface and have evolved structures that enable this; these are usually constructed from proteins and many of them are complex and specialized. In bacteria, these protein molecules are known as adhesins and are often but not always delivered at the end of long proteinaceous organelles called pili or fimbriae. The precise amino acid sequence of the adhesin, and hence its structure, can dictate which host and even which tissues within the host the bacterium sticks to and can, therefore, play a major role in dictating host range and tissue tropism. For example, enterotoxigenic *Escherichia coli* expressing K88 fimbriae will stick to piglet intestine and cause disease, those expressing K99 will stick to calf and lamb intestine, and those expressing colonization factor antigen (CFA) I and

CFAll will stick to human intestine. Similarly, *E. coli* expressing P fimbriae (otherwise known as PAP pili) will stick efficiently to the human urinary tract and cause infection at that site. After initial loose adherence, enteropathogenic *E. coli* will stick more firmly to the intestinal surface via the nonfimbrial adhesin, intimin. The receptor for intimin on the host cell surface is a protein called Tir, which is itself an *E. coli* protein that has been translocated into the host cell via a specialized needle-like structure, a type 3 secretory system (T3SS), which is itself closely related to bacterial flagella. This complex, coordinated series of events gives an insight into the extraordinary sequences that have evolved to enable bacteria to exploit their hosts as ecological niches. Viruses also rely on surface structures for host specificity, an example being influenza virus. Among several other mechanisms, the host range and tissue tropism of influenza virus is dependent on the structure of its haemagglutinin molecule. On the respiratory epithelium, haemagglutinin binds to sialic acid which is linked to galactose on the host cell surface via either an $\alpha 2,3$ or an $\alpha 2,6$ linkage. Human influenza viruses bind preferentially to the $\alpha 2,6$ -linked molecule, which is abundant on human tracheal epithelium, whereas avian influenza viruses bind preferentially to the $\alpha 2,3$ -linked version, which is abundant on duck intestinal epithelium. The different binding capacities of the haemagglutinin molecules are also important in the transmissibility of the virus, which is clearly a major element in determining whether or not an epidemic will occur. Interestingly, pig trachea expresses plenty of both types of molecule, which may explain in part why pigs are susceptible to both avian and human influenza viruses, although their distribution is different at different levels of the respiratory tract. An important nuance is that different viruses must bind to and infect the same cell in order for reassortment to result. While this raises significant questions for dogma that the pig may be a mixing vessel, it is interesting that the pig is the host of many viruses that have reassorted within swine, including the 2009 human pandemic virus. The precise cell tropism in the human respiratory tract for viruses of different host origin might also correlate with the type of disease caused, and possibly the amount of virus shed, leading both to different disease severities and potentially different transmission dynamics. Once established at a surface, pathogens have a wide array of possible strategies. They can stay at that surface and cause very little damage, and indeed be carried without causing any clinical signs. Bacteria such as *Haemophilus influenzae* and *Neisseria meningitidis* are good examples of this. Only as a result of some unknown and rare set of circumstances will these bacteria move into the bloodstream to cause septicaemia and sometimes meningitis. To survive and spread in the blood, bacteria have evolved a range of molecular strategies to inhibit the activation and activity of complement, and to avoid or resist phagocytosis. Alternatively, the bacteria can stay at the mucosal surface and cause considerable damage—by direct invasion and destruction of the tissue, by inducing a damaging inflammatory response, or by elaborating a toxin. The precise pathology caused, and consequently the clinical signs that ensue, depends on the precise nature of the toxin and the site at which it has its effects. Thus, enterotoxigenic *Escherichia coli* makes labile toxins and stable toxins, which will usually result in watery diarrhoea, whereas enterohaemorrhagic *E. coli* can make Shiga toxin, which is spread systemically and acts at a distance from the gut with severe consequences such as thrombocytopenia and kidney damage, leading to haemolytic uraemic syndrome. Other bacteria invade and spread systemically, finally lodging in particular tissues and causing direct pathology or inducing inflammatory responses that result in immunopathology (e.g. the lesions associated with systemic salmonella infections such as typhoid fever). These pathologies often result from the binding of host receptors (pattern recognition receptors, PRRs) to relatively invariant structures on the invading organisms (pathogen-associated molecular patterns, PAMPs), such as endotoxin, peptidoglycan, flagella, or in viruses' double-stranded RNA, leading to expression of a range of

cytokines that mediate the inflammatory response. If this process gets out of control, or happens at the wrong time and in the wrong place, severe pathology can result. An example of this is the systemic inflammation that leads to sepsis, with attendant tissue damage, circulatory collapse, and often death. Similarly, viruses bind to specific host cell receptors and the distribution of these receptors can determine the

8.1.1 Biology of pathogenic microorganisms 653 range of pathologies associated with the infection. For example, the henipaviruses bind to Ephrin B2, a specific N-methyl-D-aspartate (NMDA) receptor that has a particular distribution, including in the central nervous system, which underlies the preponderance of encephalitis as a clinical manifestation of henipavirus infection in humans. The receptor binding of influenza viruses is considered earlier. The broad range of cellular tropism of filoviruses, such as Ebola virus, which is mediated through viral glycoprotein spikes, is also explained by the wide cellular distribution of the heparan sulphate proteoglycans and T-cell immunoglobulin and mucin domain 1 (TIM-1) glycoproteins to which this virus can bind. Each of the different stages of bacterial infection relies on the pathogen being able to adapt physiologically and metabolically to the different niches in which it finds itself, and having the appropriate structures to survive the onslaught of innate and adaptive immune responses. It is becoming increasingly apparent that many bacteria can adapt gene expression profiles rapidly, and have sophisticated molecular mechanisms for rapid switching of many of the structures that are required for virulence or are recognized by the immune system. Virulence factors versus fitness factors Almost any gene product that has been identified as being required for infection has been called a 'virulence factor' in the literature. However, this is imprecise and can be misleading. For bacteria, many of the genes required for host exploitation might be better considered as 'fitness factors', but are no less important in the consideration of infectious diseases and how to combat them. Bacteria can often grow outside their hosts and so not all their genes are necessarily required for fitness inside the host. Those that are include well recognized virulence factors such as adhesins and toxins, and also various metabolic pathways that enable the bacterium to survive for long enough and grow in the host to cause damage. Viruses, on the other hand, are obligate host parasites. They tend (with notable exceptions such as herpesviruses and poxviruses with genomes of 100–200 kb) to have rather small genomes with few genes. Therefore, in most viruses, each gene is required for exploitation of the host in some way and is highly likely to be a fitness factor in the sense of evolutionary fitness. It may well be appropriate to consider them as virulence factors in pathogenesis. In considering virulence vs. fitness in evolution, we might ask, 'Why do pathogens cause damage rather than simply existing in harmony with their host?' This question might be framed better as, 'What evolutionary pathway has resulted in pathogens that cause damage to their hosts?' There are many possible answers. The pathogen might have evolved to exploit a particular ecological niche rather than a particular host, but has found itself by accident in a host, which it then damages almost as a bystander event. Another answer might be that by inducing a certain pathology the pathogen liberates more nutrients for itself and/or facilitates its transmission to another host (preferably in most cases before it kills its original host). Whatever the truth is behind these evolutionary pathways, it is essential that people working with infectious diseases should recognize that there is more to the evolution of a pathogen than the acquisition of a toxin or two. Indeed, in pathogens that have evolved to infect a single host, such as *Mycobacterium tuberculosis*, *Salmonella Typhi*, or *Bordetella pertussis*, among others, comparison at the whole genome level with close relatives that have retained the capacity to infect multiple hosts suggests that gene loss relative to a recent common ancestor is a common feature of host adaptation.

Adaptation to the environment A major shift in the minds of infectious disease researchers in recent years has been the realization that pathogens are far from the relatively static entities they were once thought to be. It is now clear, from many different examples, that bacterial pathogens sense the environment in which they live, and alter gene expression profiles accordingly to enable exploitation of and survival in that environment. For example, a food-poisoning bacterium such as *Salmonella enterica* might be living on a nutrient-rich piece of meat, but at a cold temperature. The meat might then be cooked, providing the bacterium with heat stress. If the meat is undercooked the salmonellae will survive the heat stress by expression of different heat-shock operons, which incidentally might also lead to the expression of genes required to survive subsequent assaults in the host. On entry into the mouth, defences such as lysozyme and IgA must be overcome, and on entry into the stomach, a very low pH is encountered. Gene expression will again change in the salmonellae such that genes for acid tolerance and acid resistance are now to the fore. Once the bacteria exit the stomach, the pH will change again, and they will be assaulted by bile salts and many other defence mechanisms until they arrive at their point of attachment to the small intestine. Although there are very few experimental data about these phenomena in actual host animals, experiments in vitro and a few experiments in cells or in animal models are beginning to reveal the complex changes that must take place in gene expression for a bacterium to establish itself in a host animal. Many of these changes are orchestrated through well-understood environmental sensory and signal transduction systems. One of the most common is the two-component sensory system. One component is a membrane protein that senses external environmental cues and the second is an intracellular protein that binds to DNA and either activates or represses the transcription and expression of sets of genes, usually called regulons. A signal is transmitted from the sensor to the activator/repressor when the environment changes. Signal transduction is achieved via histidine protein kinases. These two-component systems are very common in bacteria. Those bacteria that can live in numerous environments tend to have many more of them (e.g. c.90 in *Pseudomonas*), whereas those bacteria that have become adapted to a lifestyle in a particular host have very few (e.g. 2 in *Chlamydia*, 1 in *Mycoplasma*) and there is often a concomitant loss of genomic size. A better understanding of how bacteria behave and of the genes that are actually expressed inside the host will very likely lead to breakthroughs in the design of new antimicrobials and vaccines. This is becoming an ever more important imperative, with major concern being expressed by medical and veterinary professionals, scientists, and politicians alike about the rapidly increasing prevalence of bacteria that have evolved to become resistant to the current set of antimicrobial drugs. There is a suggestion that the world might be on the brink of returning to a 'pre-antimicrobial' era, with different bacterial infections becoming untreatable, and there are indeed

654 **SECTION 8 Infectious diseases** examples of bacteria that are resistant to all known antimicrobial drugs. A further consequence of resistance to antimicrobials is that many of our current therapies for a range of diseases, such as treatment for cancer, and major joint surgery, require extensive coverage by antimicrobial drugs so that secondary bacterial infections do not occur. If resistance continues to increase in prevalence, these therapies will also be severely compromised. **Interaction with the immune system:**

Antigenic variation The survival of pathogens in hosts is made particularly challenging by the existence of the immune system. Pathogens have responded to this challenge by evolving many specific mechanisms for the avoidance, evasion, or subversion of both innate and acquired host resistance mechanisms. For example, some viruses are inherently genetically unstable. The

natures of the polymerases that replicate the RNA genomes of influenza virus and lentiviruses such as HIV result in variants being produced at a high rate. Many variants will be incompatible with the continued existence of the virus as an entity capable of reproduction. Consequently, many defective viruses can be detected, although they are usually missed by conventional consensus-based sequencing approaches. Genetic changes may, however, not effectively alter the functionality of the viral protein other than to alter antigenicity and this may confer fitness benefits in the face of population-level immunity, leading to selection of the variant viruses. In this way, over time, progressively varying viruses evade the immunity that develops in response to infection. Some viruses, such as influenza, have evolved segmented genomes. If two viruses happen to be occupying the same host cell, different segments can rearrange, and a new virus can be assembled. This type of large-scale reassortment leads to the so-called 'antigenic shifts' that allow invasion of populations entirely immunologically susceptible to new variants, as occurred with the 2009 swine influenza H1N1 pandemic. At an entirely different level, within-host antigenic variation is thought to be one mechanism that allows HIV to continue to be carried chronically in the face of what might appear to be a strong immune response. Many other pathogens may also escape the immune response by antigenic variation within the host. Bacteria such as *Haemophilus influenzae*, *Neisseria meningitidis*, and *Campylobacter jejuni* have evolved tracts of repetitive DNA in single base pair repeats or repeats of four or more base pairs. The number of these repeats can change, apparently randomly. This is a powerful mechanism that allows the existence of a population of bacteria with several different antigens that may be 'randomly' expressed or not expressed in individuals within the population. This might be a kind of altruistic evasion strategy whereby some members of the population are lacking a particular structure which is itself a target for the immune system, such that they will survive and thus continue the existence of that bacterial population. Other mechanisms involving recombination and gene conversion exist in other pathogens, leading to the expression of alternative antigenic versions of the same protein and underlying the cycling of different forms of, for example, variant surface glycoprotein in trypanosomes and the opportunistic fungal pathogen *Pneumocystis jirovecii* or pili in neisseriae. Pathogens have also evolved mechanisms to subvert the immune system by mimicking elements of the innate response. Good examples are herpes and poxviruses that encode chemokine homologues and/or chemokine receptor homologues or analogues. Genomes Many bacterial, viral, fungal, and protozoal genome sequences are now complete. Genome sequencing technologies are being used extensively to understand pathogens in detail and in completely new ways. The availability of genome-scale sequence data for these pathogens has revolutionized our understanding of their biology and has opened up completely new methods of study and ways of thinking about how they interact with their hosts. Immediate benefits of having complete genome sequences include the obvious knowledge of the complete gene set. This means that we now 'know' every conceivable target for the immune system and every conceivable target for novel antimicrobial development. The real challenge for researchers and infectious disease physicians is to sift and unravel the whole mass of information and to select from it that which is genuinely useful. It might be better to invent strategies to let the host biology and the genome itself indicate which genes and antigens are likely to be useful as vaccine targets. Some of these methods are now being published and a good example is 'reverse vaccinology'. Here, genes for outer membrane proteins from a pathogen of interest are selected using computer algorithms, cloned using the polymerase chain reaction or synthesized de novo, and the encoded protein expressed and purified. These proteins can then be used to interrogate sera from animals or humans that have been infected and are convalescent, to identify which of them is expressed as an antigen

during infection, although this step is not essential. The proteins can subsequently be used to immunize animals with the intention of testing the resultant immune response for its ability to protect against virulent challenge in different infection models. The choice of read-out and model is of course crucial if an effective vaccine for humans is to be designed. Despite many possible pitfalls, reverse vaccinology is an exciting technology platform with great promise for the exploitation of genomes to generate completely new candidate vaccines against bacteria. Indeed, a vaccine against group B meningococcus has been developed and licensed for use in children based on this approach. Another fascinating story, emerging from the availability of many genome sequences derived from field isolates of bacteria has been the recognition of diversity within bacterial species and the evolutionary relationships between bacteria that this implies. It is clear that many bacterial species share their DNA promiscuously and that this can lead to rapid evolution of drug resistance and altered pathogenicity. Many tried and trusted schemes for classifying and typing bacteria need to be reassessed in the light of genomic information. Highly used typing schemes, such as the Kaufman–White scheme for salmonellae, based on recognition of antigens on the bacteria by standardized antibodies, are being replaced by DNA-based methods such as multilocus sequence typing, analysis of single nucleotide polymorphisms, and very high throughput sequencing based approaches; the last of these will become the method of choice for many diagnostic applications once even newer sequencing technologies become established. DNA sequencing technology is improving at an

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astonishing rate, and ultra-high-throughput sequencing machines are now available that make the determination of a draft genome for a bacterium less expensive than a routine microbiological workup. Real-time whole genome sequencing is already at a stage where it could be used to inform infection control measures. A study of *Staphylococcus aureus* in which 63 isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were sequenced gave clear-cut information about the geographic origin of the isolates and definitive information about person-to-person spread in the hospital environment. A similar study on *Streptococcus pneumoniae*, in which 240 genome sequences were determined, has been able to follow how the bacteria have adapted to clinical interventions, in the form of vaccines and antimicrobials, over very short time scales. This kind of approach is becoming routine in hospitals and is revolutionizing how we think about infections and their control. Genome sequencing approaches are also able to address questions of global importance, tracing the epidemiology of infectious disease outbreaks in time and space. For example, sequencing of isolates of *Vibrio cholerae* from around the globe has shown that the seventh pandemic clone of this organism originated in the Bay of Bengal and spread round the world, in at least three distinct waves. The outbreak of cholera in Haiti, beginning in 2010, became a politically charged event; and whole genome sequencing was able to show unequivocally that rather than this outbreak being local and triggered by the earthquake that led to the breakdown of public health measures, the pathogen involved was actually derived from an organism prevalent in Nepal. This study also gave a clear glimpse into the role that international travel and globalization can play in altering local microbiological events. Genome sequencing will be a major tool in understanding and facing the challenge provided by the increasing rates of antimicrobial resistance, mentioned earlier. Whole genome sequencing of clinical isolates is being used to identify the presence of genes encoding resistance to antimicrobials, and the arrangements of those genes on different mobile genetic elements. This opens up new epidemiological studies, and new understanding of how bacteria, and indeed their genes, move between humans, other animals, and the environment outside the host. Future challenges Most infectious diseases are unlikely to be eradicated in the near future. Even if

they were, new infectious agents would inevitably evolve to exploit the rich environments presented by host animals. Infectious disease biology and medicine are currently undergoing a renaissance, driven by the revolution in genome science, allied to the real and present dangers still presented by many pathogens, and their evolution to become resistant to the drugs used to treat the diseases caused by them. They have the capacity to deliver sudden, severe global pandemics resulting in high global mortality. Emerging infections presenting acute public health problems are likely to be viral in nature, to have originated in an animal population and, therefore, at least initially, to be zoonoses, and to be spread quickly via air travel. Changing social conditions (e.g. increasing urbanization in certain parts of Africa), bring together animal and human populations that have rarely if ever been closely associated on a large scale. This brings with it an increased chance for microorganisms to be shared between these species, with a resulting increased chance of new pathogens emerging. The devastating effects of Ebola in West Africa from 2013 to 2016 exemplify this perfectly. Even relatively minor changes in societal behaviour can lead to major disease problems. For example, changes to methods for preparing cattle feed led to the emergence of the BSE prion as a human disease problem in recent years in the United Kingdom and elsewhere. This analysis does not take into account the added problem of possible bioterrorism attacks, although the impact of these has not to date met the level of concern over them. To deal with these disease threats, the regulatory framework underlying the development and legal deployment of antimicrobials and vaccines will have to be adapted and evolved in step with the evolution of the diseases themselves. The pathogens are likely to win this race too! Artificial distinctions between 'human' and 'veterinary' medicine need to be removed. Most pathogens infect more than one species of host animal and certainly do not respect the anthropocentric division of research effort. The concept of 'One Medicine', introduced by Calvin Schwabe, is nowhere more pertinent than in consideration of infectious diseases and the biology of pathogens, and is likely to be particularly important in dealing with the emerging antimicrobial resistance problem. A concerted effort is needed to deal with pathogens in all parts of the world and not just in the developed world. Inexpensive but effective intervention strategies must be developed to defeat acute and chronic infectious diseases worldwide. The lives affected by pathogenic microorganisms in developing countries are just as valuable as those in more affluent areas, but are far more numerous. A focus on primary medical care and early detection of outbreaks would be far more beneficial than high-tech approaches that some claim will enable us to predict which specific pathogens will cause future human pandemics. It remains our task, whether from a medical, veterinary, or basic science background, to try to understand how pathogenic microorganisms work, and to harness that knowledge to defeat, wherever possible and by whatever means, these ever-adaptable scourges.

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system through chemokine mimicry. Nat Immunol, 2, 116–22.

Revision #1

Created 2026-01-22 16:45:02 UTC by Omar Ayman

Updated 2026-01-22 16:45:02 UTC by Omar Ayman