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Chapter 1

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Nature and pathogenicity of micro-organisms

Human existence would be impossible without the micro-organisms that surround us, as they play critical roles in processes as diverse as photosynthesis, nitrogen fixation, production of vitamins in the human intestine and decomposition of organic matter. They are the sole, true 'recyclers' of our planet. Micro-organisms are also the major driving force behind the evolution of life. They evolved photosynthesis and respiration, which have since been acquired by present-day eukaryotes, and they mediate genome rearrangements in infected host cells.

In a rather simplified view, micro-organisms may be considered to be no more than 'little machines that multiply'. In fact, this is what they do best. We are starting to understand some of the strategies micro-organisms have developed to stay alive, grow and reproduce. The lifestyle of a micro-organism is intimately related to its environment, whether that environment is the human body or a polluted riverbed. Some highly specialized micro-organisms have adapted to the harsh environmental conditions, while others, such as root-colonizing bacteria and our own intestinal flora, have taken advantage of the abundant resources provided by higher organisms.

This chapter focuses on the lifestyle of pathogenic micro-organisms and how they infect us, reproduce and cause disease. We shall use the word 'pathogenicity' to indicate the capacity to cause disease (or damage) in nonimmune individuals. Although the word 'virulence' is often used in the same sense, we mean it to refer to the severity of the illness that is caused. Communicability refers to the transmissibility or infectiousness of micro-organisms.

DEFINITION AND COMPARISON OF INFECTIOUS AGENTS

The definition of an 'infectious agent' was proposed by J Henle in 1840 and put to the test by the German physician Robert Koch. In 1876, Koch reported experiments on mice with *Bacillus anthracis* showing that:

- a single micro-organism could be isolated from all animals suffering from anthrax;
- the disease could be reproduced in an experimental host by infection with a pure culture of this bacterium; and
- the same micro-organism could subsequently be reisolated from the experimental host.

This definition is an oversimplification because many pathogenic microbes have never been cultured (e.g. *Mycobacterium leprae*), others lack a suitable animal host in which the infection can be reproduced (e.g. *Salmonella enterica* serovar Typhi) and some cause disease only under specific conditions.

Infectious agents can be divided into four groups:

- Prions, which consist of only a single protein (PrP). The infectious form (PrP^{TSE})[1] is transmissible as spongiform encephalopathy (see Chapter 22).
- Viruses, which contain proteins and nucleic acids, and virioids which contain only nucleic acid. These characteristically disassemble after cell entry and then assemble their progeny during replication[2] (see Chapters 151 and 152).
- Bacteria, including archaea and eubacteria. Unlike eukaryotes, the DNA genomes of these prokaryotes are not separated from the cell by a membrane. Unlike viruses, they remain enclosed within their own cell envelope throughout their life cycle (see Chapters 165–177).
- Eukaryotes, including fungi (see Chapters 178 and 179), protozoa (see Chapters 180–183) and multicellular parasites (see Chapter 184). These organisms have subcellular compartments, including the nucleus, where DNA transcription occurs.

Table 1.1 compares the properties that define prokaryotes with eukaryotes and Table 1.2 emphasizes the differences between bacteria and fungi, many of which determine the specificity of antimicrobial agents.

GENERAL PROPERTIES AND CLASSIFICATION OF VIRUSES

Taxonomy of viruses

The hierarchical classification system[4] groups viruses in families (-viridae), genera (-virus or -viruses) and species (-virus), emphasizing similarities in the type (DNA or RNA) and nature (single stranded or double stranded, segmented or nonsegmented) of genetic material, and structural features (size, symmetry and presence or absence of a lipid envelope; see Table 1.3). For example, picornaviridae is a family of small, non-enveloped RNA viruses containing the enterovirus genus, which in turn includes poliovirus species of serotypes 1, 2 and 3.[5] The Baltimore classification system[6] emphasizes the relationship of the genetic material of the virus and the viral replication scheme. For example, group IV contains viruses with single-stranded RNA genomes where the mRNA shares the same sense as the viral RNA (+ssRNA), which includes picornaviridae, enterovirus and poliovirus.

Common steps in the replication of viruses

Virus replication involves the following steps.

1. Attachment: Virus particles (virions) must first attach to specific receptor(s) on the surface of a host cell.
2. Penetration: This may proceed via direct fusion with the cell membrane or through endocytosis and pH-mediated fusion.
3. Uncoating: The virion disassembles, freeing nucleic acid and viral proteins needed for replication.

Table 1.1 Comparison of prokaryotes and eukaryotes

Feature	Prokaryotes	Eukaryotes
Chromosome	Single, circular or linear	Yes
Gene organization	Operon-polycistrionic mRNA	Single genes and block of genes
Nucleosomes	No	Yes
Nuclear membrane	No	Yes
Mitosis	No	Yes
Introns in genes	No	Yes
Transcription	Coupled with translation	Separate from translation
mRNA	No terminal polyadenylation (except archaebacteria); polygenic	Terminal polyadenylation; usually monogenic
First amino acid	Unstable formylmethionine (except archaebacteria)	Methionine
Ribosome	70S (30S + 50S)	80S (40S + 60S)
Cell wall	Presence of muramic acid, D-amino acids, peptidoglycan (except archaebacteria and mycoplasma)	No muramic acid, D-amino acids or peptidoglycan
Membrane	No sterols or phosphatidyl-choline (except mycoplasma)	Sterols and phosphatidyl-choline
Endoplasmic reticulum	No	Yes
Mitochondria	No	Yes (*E. histolytica*, *Giardia* and microsporidia have vestigial remnants of mitochondria)
Lysosomes and peroxisomes	No	Yes
Movement	By flagella, composed of a single fiber	Ameboid, by cilia or cilia-like flagella

Table 1.2 Comparison of bacteria and fungi

Characteristics	Bacteria	Fungi
Cell volume (mm^3)	0.6–5.0	Yeast: 20–50; molds: greater than yeast
Nucleus	No membrane	Membrane
Mitochondria	No	Yes
Endoplasmic reticulum	No	Yes
Sterol in cytoplasmic membrane	No (except for mycoplasma)	Yes
Cell wall components	Muramic acids and teichoic acids; no chitin, glucans or mannans	Chitin, glucans and mannans; no muramic acids or teichoic acids
Metabolism	Autotropic or heterotropic	Heterotropic
Sensitivity to polyenes	No	Yes

Adapted from Kobayashi.[3]

4. Replication: Viral proteins and messages are expressed. Intermediates such as viral complementary RNA or integrated proviral DNA may be needed.
5. Assembly: New virions containing viral nucleic acid are formed.
6. Release: New virions are released from the cell via lysis of the cell or intra- or extracellular budding.

Structure of viruses

The virion is designed to protect the viral genome and to mediate the migration of the virus and the invasion of the target host cell. Viruses are small, the smallest being ~25–30 nm in diameter, while the largest (mimivirus, an infectious agent of amebae) are 400 nm or more in size.[7] The viral genome is tightly associated with a nucleoprotein in a highly organized core structure, the nucleocapsid. In some virus families, such as (–) strand RNA viruses and retroviruses, the virion contains enzymes required for early stages of virus replication. The main proteinaceous outer structure of a virus is the capsid or tegument. Clefts or vertices in the assembled capsid, protruding protein spikes or other structures may mediate viral attachment. Alternatively, the capsid is surrounded by an outer lipid membrane (the envelope) derived from membranes of the host cell in a process termed 'budding.' Viral proteins inserted within the envelope then serve as receptors for specific host cell molecules.

The viral genome

Viral genomes consist of DNA or RNA, and range from 1.7 kb to 1.2 Mb in size. There may be only a single gene in the smallest virions, whereas the larger genomes encode hundreds. For example, parvoviridae have only two open reading frames, whereas the vaccinia poxvirus has 263 known genes.

The nucleic acid of all mammalian DNA viruses except the parvoviridae and circoviridae is double stranded (dsDNA, Fig. 1.1). In contrast, the nucleic acid of all mammalian RNA viruses except the reoviruses (e.g. rotavirus, coltivirus) is single stranded (ssRNA). The genome structure may be linear, circular or rod-like, and either non-segmented or segmented. Genome segmentation facilitates genetic exchange between co-infecting virions in a process known as reassortment. Some viral nucleic acid molecules may contain modified nucleotides, which inhibit host cell nucleases and/or mediate recognition by viral polymerase. Linear genomes often contain conserved terminal sequences. When complementary, these allow partial circularization of the genome or formation of panhandle or tube-like structures. Terminal sequences may also allow incomplete replication products to recombine or mediate recognition by proteins that prime transcription or replication. The proviral DNA of retroviruses is flanked by repeat sequences similar to transposable genetic elements.

The genome of ssRNA viruses may have either of two possible polarities. The viral RNA (vRNA) of positive-strand RNA viruses acts directly as mRNA for protein synthesis; they resemble eukaryotic RNAs with a cap at the 5′ end and are polyadenylated at the 3′ end. In contrast, the RNA-dependent RNA polymerase of (–) ssRNA viruses uses the vRNA as a template for mRNA transcription. Negative-strand RNA genomes have neither cap structures nor poly-A tails, often parasitizing cap structures from cellular pre-mRNA or mRNA. Retroviruses synthesize a dsDNA copy of the positive-strand RNA genome, which integrates into the cellular DNA and then expresses viral messages.

Table 1.3 Classification of viruses

Family name	Example	Genome size (kb) polarity (+ or –) and segments	Morphology	Envelope
DNA viruses				
Single-stranded (Class II)				
Parvoviridae	Human parvovirus B19	5 (±) single	Icosahedral	No
Mixed-stranded (Class VII)				
Hepadnaviridae	Hepatitis B	3 (±) single	Icosahedral	Yes
Double-stranded (Class I)				
Papovaviridae	Wart virus	8 (±) single	Icosahedral	No
Polyomaviridae	JC virus	5 (±) single	Icosahedral	No
Adenoviridae	Adenovirus	36–38 (±) single	Icosahedral	No
Herpesviridae	Herpes simplex	120–220 (±) single	Icosahedral	Yes
Poxviridae	Vaccinia	120–280 (±) single	Complex	Yes
RNA viruses				
Positive-sense (Class IV)				
Picornaviridae	Poliovirus	7.2–8.4 (+) single	Icosahedral	No
Togaviridae	Rubella	12 (+) single	Icosahedral	Yes
Flaviviridae	Yellow fever	10 (+) single	Icosahedral	Yes
Coronaviridae	Infectious bronchitis	16–21 (+) single	Helical	Yes
Negative-sense (Class V)				
Rhabdoviridae	Rabies	13–16 (–) single	Helical	Yes
Paramyxoviridae	Measles	16–20 (–) single	Helical	Yes
Orthomyxoviridae	Influenza	14 (–) 8	Helical	Yes
Bunyaviridae	California encephalitis	13–21 (–) 3	Helical	Yes
Arenaviridae	Lassa fever	10–14 (–) 2	Helical	Yes
Filoviridae	Marburg, Ebola	19 (–) single	Helical	Yes
Reverse (Class VI)				
Retroviridae	HIV-1	3–9 (+) diploid	Icosahedral	Yes
Double-stranded (Class III)				
Reoviridae	Rotavirus	16–27 (±) 10-12	Icosahedral	No

The capsid

The viral genome is protected by one or more protein coats, the nucleocapsid and/or capsid. The capsid is made of structures known as capsomeres, which consist of proteins coded by the viral genome, and accounts for a large portion of the viral mass. Papillomavirus produces only two capsid proteins and poliovirus four, but more complex viruses may encode a much larger variety.

Picornaviruses, adenoviruses and papovaviruses have a nucleocapsid structure with icosahedral symmetry. The capsid consists of 20 triangular facets and 12 corners or apices. Influenza, measles and rabies virus form capsids with helical symmetry. The central core is formed by the nucleic acid genome, around which the nucleocapsid proteins are arranged like the steps of a spiral staircase, forming long cylinders (Fig. 1.2).

More complex virion morphologies also exist. Bacteriophages, which use bacteria as hosts, have additional attachment structures fixed to the capsid. The nucleocapsid of orthopoxviruses, such as variola and vaccinia virus, consists of a network of tubules, sometimes surrounded by an envelope, forming a brick-shaped virion.

The envelope

Enveloped viruses contain nucleocapsids of either icosahedral (e.g. herpesviruses, togavirus) or helical symmetry (e.g. influenza). The outer envelope consists of a lipid bilayer derived from host cell membrane in which the viral glycoproteins are embedded. The viral matrix proteins (M proteins) associate with the envelope, playing an important role in the structural organization of the virion by connecting the capsid to the viral glycoprotein inserted in the lipid bilayer. Besides oligosaccharide residues, the glycoproteins contain a membrane anchor and, in many cases, one or two molecules of fatty acid. Glycoproteins play a key role in the attachment of virions to the cell surface and penetration into the cell.

Some glycoproteins have enzymatic activity, such as the influenza virus neuraminidase, which promotes the release of newly formed viral particles from the host cell membrane. Maturation of protein structures and transcription steps may occur after release from the host cell. For example, the typical conical core of the human immunodeficiency virus retrovirus may be incomplete at release and partial reverse transcription occurs within the virion.[9]

Viral gene expression strategies

In the Baltimore classification, seven major viral replication strategies are distinguished (see Figs 1.1, 1.3 and the following).

In positive-strand RNA viruses (Baltimore Class IV), the viral genome serves immediately as mRNA. The first step in viral infection consists of a complete translation of the genome to produce a polyprotein, which is sequentially processed into smaller polypeptides via enzymatic cleavage which is at least partially autocatalytic. In the early phase, processing of viral structural proteins occurs slowly, due to the low concentrations of mature viral proteases. Viral complementary RNA is synthesized, followed by new viral RNA. As the viral proteases accumulate, core proteins are more efficiently processed, assemble and begin to encapsidate viral RNA. This strategy is used by picornaviruses and flaviviridae, including hepatitis C viruses. In other (+)ssRNA virus families (e.g. togaviridae, coronaviridae, caliciviridae and hepatitis E virus), the viral complementary RNA serves not only for production of

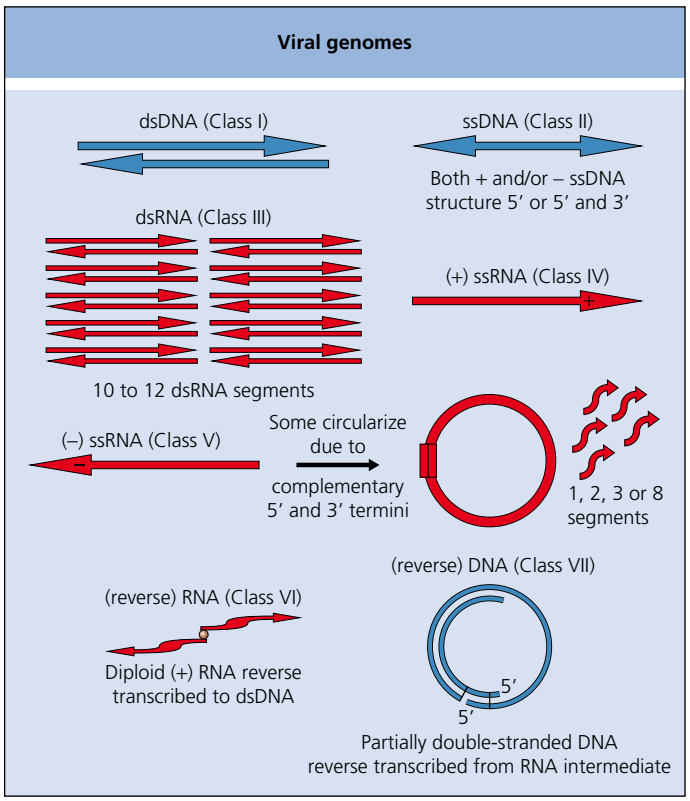


Fig. 1.1 Viral genomes. The schematic genomes are grouped according to the Baltimore classification system. DNA is represented by blue shades, RNA in red shades. Arrows indicate direction of DNA/RNA 5′ to 3′, not necessarily relationship of sense of messenger RNA to that of genome. See Figure 1.2 for a diagram of typical viral structures, Table 1.5 for a list of representative viruses within groups and Figure 1.3 for strategies of viral replication.

full length vRNA, but also subgenomic transcripts encoding structural proteins, allowing regulation of expression.

The RNA-dependent RNA polymerase of (–)ssRNA viruses (Baltimore Class V) must first produce transcripts using the infecting vRNA as a template. Primary transcription may generate a full-length viral complementary RNA (positive-strand) which acts as both mRNA for viral protein synthesis as well as a template for transcription of new viral RNA. This is typical of segmented (–)ssRNA viruses including orthomyxoviridae and most bunyaviridae. Alternatively, the incoming viral RNA may initially be transcribed into mRNA messages for individual genes, which must accumulate before transcription of full-length viral complementary RNA and replication of viral RNA can occur (e.g. paramyxoviridae, rhabdoviridae). The late phases of replication transcription and viral protein synthesis proceed simultaneously.

The arenaviridae and the some bunyaviridae use a somewhat more complicated strategy, termed ambisense. For one or more of the RNA genome segments, both the viral RNA and the viral complementary RNA serve as templates for mRNA transcription by the viral polymerase. This does not result in the formation of complementary double-stranded mRNAs, as different portions of the genome are transcribed from the viral and complementary RNA strands.

Only a few double-stranded RNA viruses (reoviridae, Baltimore Class III) produce human disease. The coltivirus Colorado tick fever virus causes a febrile syndrome with myalgia, headache, rash and occasionally encephalitis. The rotaviruses are important causes of diarrheal illness. Virions have 10–12 dsRNA segments and replication resembles that of (–)ssRNA viruses in that RNA is not infectious, and transcription of segment length mRNAs using the negative strand of the genomic dsRNA must first occur before genome replication. Regulation is accomplished via transcription rate (inverse to segment length) and efficiency of translation (varying up to 100-fold).

In the case of ssDNA viruses (parvoviridae and circoviridae, Baltimore Class II), the incoming genome is first used to express proteins that permit the synthesis of the complementary DNA strand. Double-stranded DNA is a replication intermediate, and DNA replication is dependent on repeats which form DNA structures on one or both ends. These parvoviridae include the B19 erythrovirus that causes erythema infectiosum (fifth disease) in children and exanthem, arthropathy and aplastic crisis in adults. This family also includes the apathogenic adeno-associated dependoviruses (AAV), which serology indicates cause ubiquitous human infection.[10] Parvoviridae have 4.5–5.5 kb ssDNA genomes and only two open reading frames, one of which codes for between two and four nonstructural proteins and the other coats polypeptides. The circoviridae have circular ssDNA genomes. This family includes Torque Teno viruses (TTV, TTMV) which cause widespread human infection without evidence of disease.[11]

Replication of dsDNA viruses (Baltimore Class I) proceeds to lysis or latency depending on cellular conditions. The lytic phase can be subdivided into early and late phases. In the early lytic phase, viral genes alter cellular conditions to allow efficient viral DNA synthesis and transcription, often activating the host cell and inducing cell division. In the late lytic phase, viral proteins accumulate, virions are assembled and released upon death of the cell. In latency, viral gene expression is confined to functions that prevent replication while maintaining the viral genome within the cell, often for the lifetime of the individual. When cellular conditions become favorable, the latent virus can be 'activated' into lytic replication.

There are many dsDNA viruses of medical importance for humans, including:

- herpes simplex viruses (cause of genital and labial herpes, meningitis and encephalitis);
- varicella-zoster virus (the causative agent of chickenpox);
- human cytomegalovirus (frequently causing disease in immunocompromised hosts);
- Epstein–Barr virus (causing infectious mononucleosis);
- adenoviruses (causing respiratory disease and conjunctivitis);

and many others.

In the case of the retroviruses (Baltimore Class VI), the virus enters the cell and uncoats, discharging the preintegration complex, consisting of the polyadenylated, diploid viral RNA genome together with nucleoproteins, the viral reverse transcriptase and the viral integrase. The two RNA genomes are converted to a single, mostly dsDNA copy by reverse transcriptase in a process requiring template switching. The viral integrase then cuts the host genome and inserts the linear viral DNA into the chromosome as a provirus. This process may require cellular activation and/or cell division, though retroviruses can persistent for weeks at stages before integration. Integrated virus may become latent, with limited or no transcription by cellular RNA polymerase II, until conditions allow virus replication.

More complex retroviruses (e.g. spumaviruses, lentiviruses) first transcribe multiply-spliced mRNAs that direct the synthesis of regulatory proteins. As these accumulate, the processing of viral transcripts changes and more singly or unspliced mRNAs coding for viral structural proteins are produced. For example, human immunodeficiency virus (HIV) *Rev* protein, produced from early, multiply-spliced RNA transcripts, prevents splicing and allows nuclear export of singly spliced and unspliced messages.

Hepadnaviruses (Baltimore Class VII), including hepatitis B virus, encode genetic information in DNA but use reverse transcription during infection in the cell to produce the (–) strand of viral DNA, which in turn is used as a template for synthesis of (+) strand viral DNA.

GENERAL PROPERTIES AND CLASSIFICATION OF BACTERIA

Bacteria are small (0.6–4.0 µm) unicellular organisms; 3×10^{12} bacteria weigh in the order of 1 g. Under optimal conditions, a bacterium may divide between two or three times per hour. Theoretically, nearly 300 g of bacterial mass can be produced from a single bacterial cell in

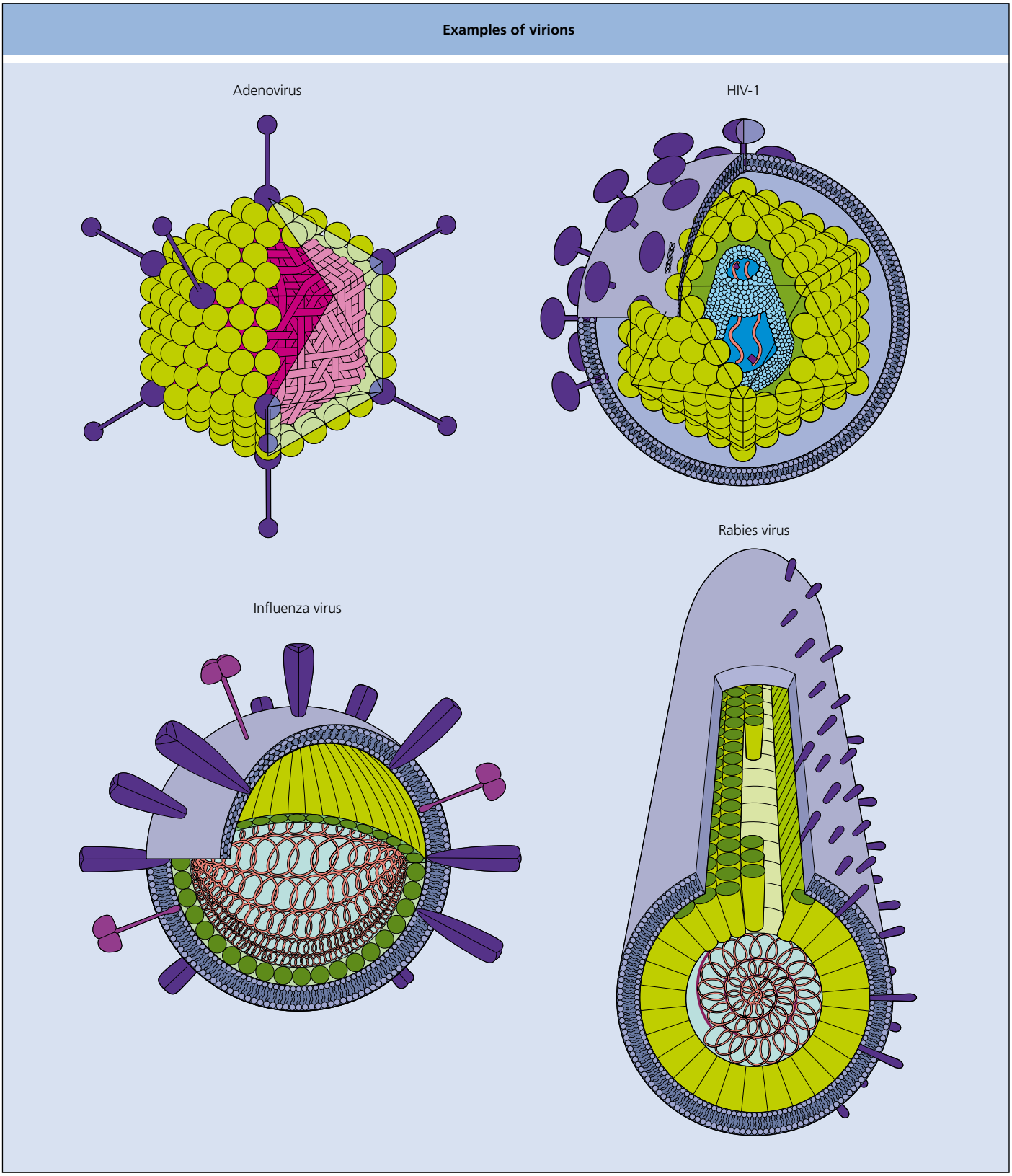


Fig. 1.2 Examples of virions. Adenovirus is an icosahedral DNA virus without an envelope; fibers extend from the 12 points of the icosahedral coat; DNA forms a ribbon-like molecule. Approximate size 8 nm. HIV-1; glycoprotein (GP) molecules protrude through the lipid membrane; the icosahedral capsid encloses a truncated conical nucleocapsid in which the diploid RNA is enclosed. Approximate size 100 nm. Influenza virus is an enveloped RNA virus containing nucleocapsid of helical symmetry; spikes of hemagglutinin and neuraminidase protrude from the lipid bilayer. Approximate size 100–200 nm (variable). Rabies virus is a helical RNA nucleocapsid with a bullet-shaped lipoprotein envelope in which approximately 200 GPs are embedded. Approximate size 150 nm. (The diagram is not to relative scale.) Adapted from Collier and Oxford[8] by permission of Oxford University Press.

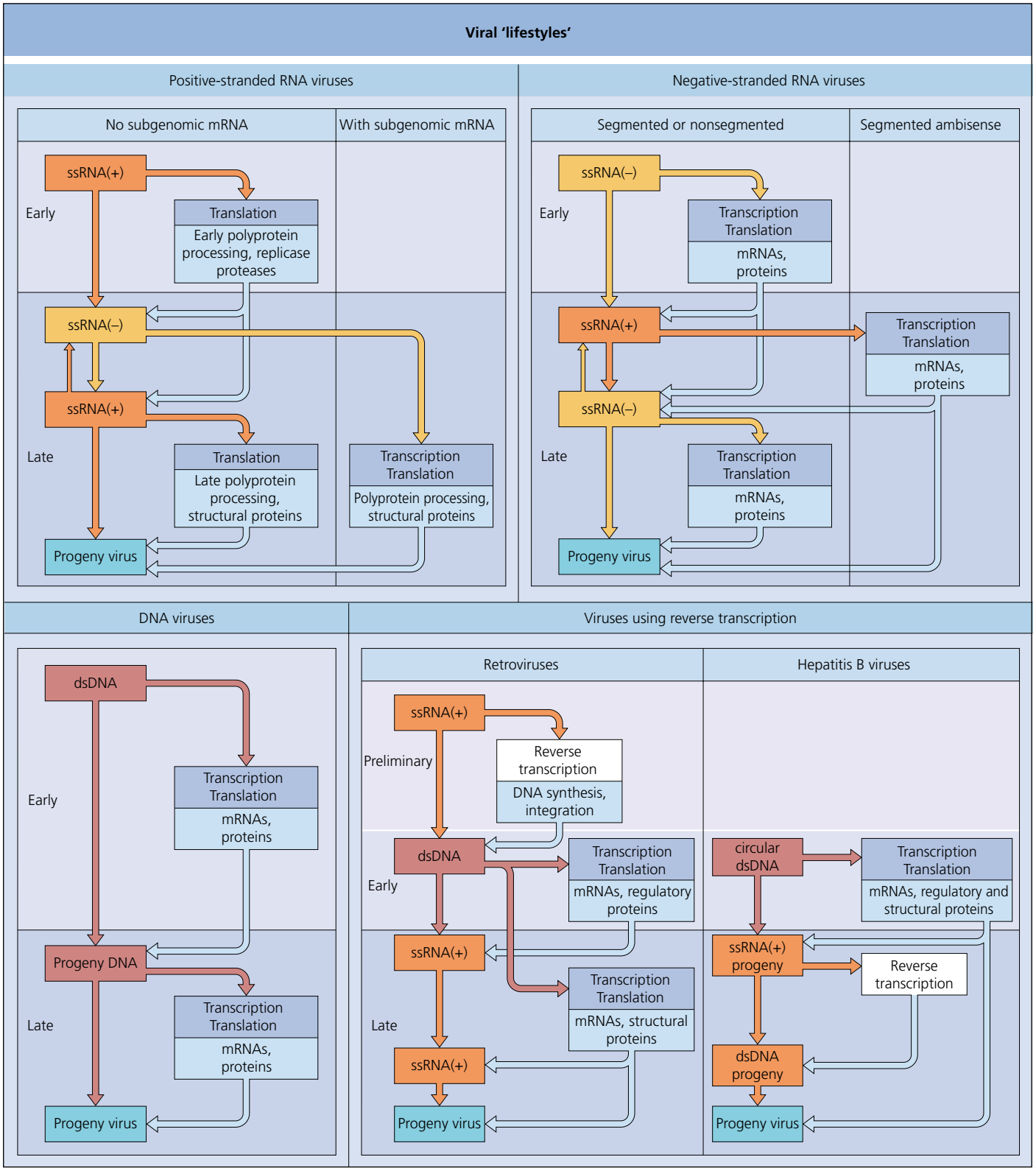
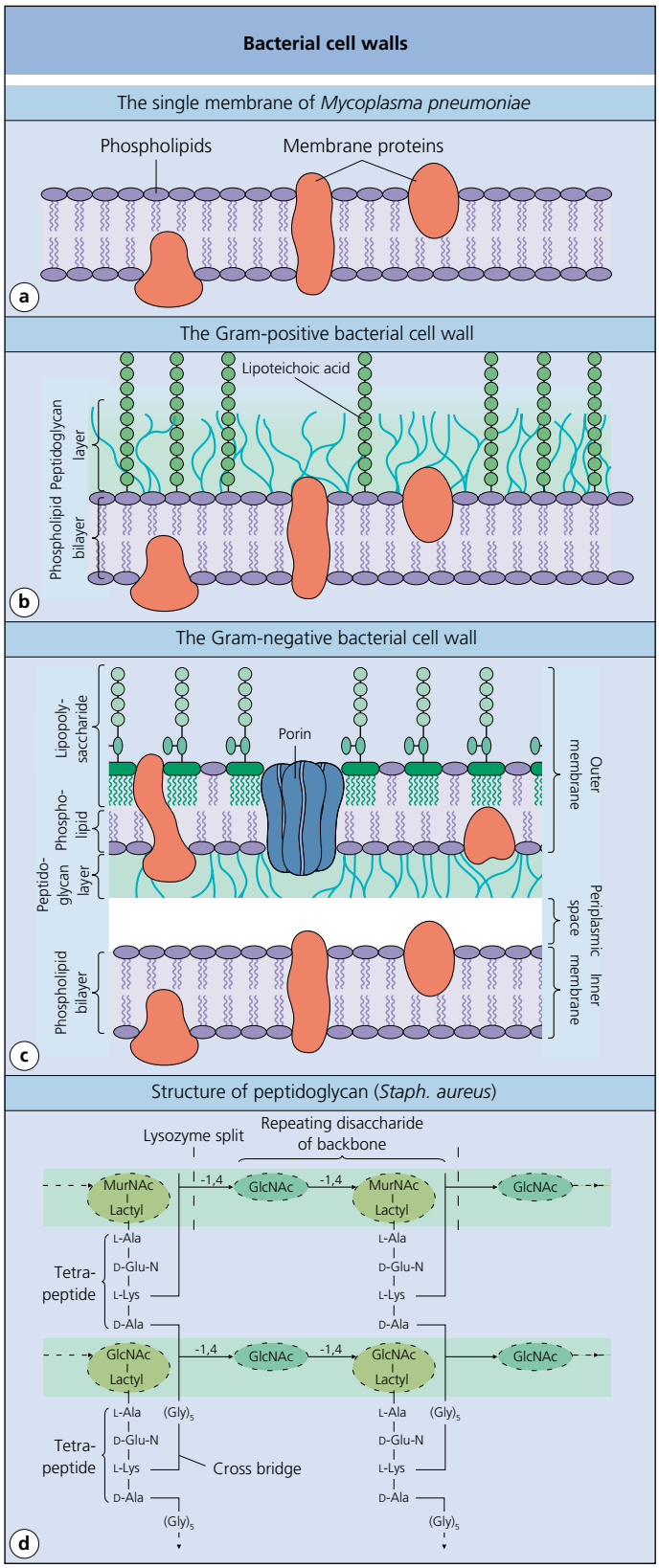


Fig. 1.3 Viral 'lifestyles'. Upper left. Single stranded (+) sense RNA viruses can be subdivided into those which produce subgenomic mRNAs (e.g. togaviridae, coronaviridae, calciviridae), allowing additional regulation of transcription, and those which do not (poliovirus, hepatitis A virus, flaviviridae including hepatitis C virus). Upper right. Negative sense RNA viruses must first transcribe RNA messages from incoming viral RNA (vRNA), as well as making a copy complementary to the viral genomic DNA (vcRNA) to serve as a template for synthesis of new viral RNA. Some (–) ssRNA viruses use both vRNA and vcRNA as templates for transcription of mRNA (arenaviruses, some bunyaviruses). Lower left. Double stranded DNA viruses have early and late lytic phases of replication, and some (e.g. Epstein–Barr herpesvirus) also have latent (usually episomal) phases. Lower right. Viruses using reverse transcription include RNA retroviruses (e.g. HIV), which reverse transcribe a dsDNA copy from diploid (+) ssRNA vRNA, and hepadnaviruses (e.g. HBV) which reverse transcribe the (–) strand of DNA from RNA transcribed from incoming viral DNA. (Courtesy of Menno Kok & Jean-Claude Pechere.)

one day. Such small organisms profit from a favorable cell surface-to-volume ratio, which allows metabolic fluxes largely superior to those attained by the larger eukaryotic cells. Bacteria react very quickly to environmental changes, regulating gene transcription to adapt their physiology.



Bacteria were probably the first cells to appear on earth more than 3.5 billion years ago. They have since developed into an overwhelming diversity representing the bulk of the world's biomass today. Although evolution has not led to bacteria associating into multicellular organisms, they are capable of cell-to-cell communication.[12] By using low molecular weight compounds, bacteria have found a way to sense how dense their local population is and decide whether or not to activate developmental programs such as plasmid conjugation, light production (in association with deep-sea fish) or virulence gene expression.

Different cell morphologies can be observed with light microscopy (e.g. spherical cocci, rod-shaped bacilli, curved vibrios, spiral treponemes). Electron microscopy unveils a distinctive cell wall, a simple nuclear body without a nuclear membrane and the presence in the cytoplasm of ribosomes and mesosomes, sometimes granules of reserve material, but no endoplasmic reticulum and no organelles such as mitochondria or chloroplasts. There frequently are appendages such as flagella that are used for motility, pili and fimbriae that may be used for adhesion or for conjugation.

Bacterial dichotomy revealed by a simple staining technique

In 1884, the Danish bacteriologist Hans-Christian Gram developed a simple staining technique that distinguishes two types of bacteria: the Gram-positive and the Gram-negative bacteria. The distinction is based on the ability of one group of bacteria, the Gram-positives, to retain a crystal violet-iodine dye in the presence of an organic solvent such as alcohol or acetone. The solvent dissolves the dye from Gram-negatives and they can be counterstained with other dyes such as safranin. This simple observation reflects distinctive structures. Gram-positive bacteria characteristically have a thick cell wall made up mainly of a vast molecule of peptidoglycan, with protruding chains of teichoic acids. Surrounding the peptidoglycan skeleton in the periplasm of Gram-negative bacteria is an additional asymmetric outer membrane, the outer layer composed of lipopolysaccharide (endotoxin) (Fig. 1.4). *Escherichia coli* is an example of a Gram-negative bacterium; it is rod shaped and growing cells are between 2 µm and 4 µm long.

The rigid cell wall determines the shape of bacteria and allows them to resist the osmotic pressure caused by the large difference in solute concentration between the cytoplasm and the environment. *Mycoplasma* spp. lack peptidoglycan and thus have neither a rigid wall nor a defined shape.

Fig. 1.4 Bacterial cell walls. (a) *Mycoplasma pneumoniae* has a single membrane, made up of phospholipids and membrane proteins. (b) In Gram-positive organisms the cytoplasmic membrane is covered with a thick layer of peptidoglycan; chains of lipoteichoic acid anchored in the cell membrane protrude outside. Negatively charged teichoic acids are covalently attached to the peptidoglycan. Cell wall proteins also are covalently attached to the peptidoglycan. There is no periplasmic space in Gram-positive bacteria. (c) The cell wall of a Gram-negative rod is more complex. The layers are: the cytoplasmic membrane, the periplasmic space, a layer of peptidoglycan which is thinner than that in Gram-positive bacteria and an asymmetric outer membrane. The inner leaflet of the outer membrane is made of phospholipids. The outer leaflet has lipopolysaccharides as its principal lipids; porins, which are channel-forming proteins often organized as trimers, allow the penetration of hydrophilic molecules through the outer membrane. (d) The peptidoglycan of *Staphylococcus aureus* has polysaccharide chains ('backbone') that are alternating residues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). Tetrapeptides are attached to MurNAc and are linked together by pentaglycines bridging the L-lysine of each tetrapeptide chain to the D-alanine of the neighboring one. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Organization of the bacterial cell

The bacterial cytoplasm does not contain physically separated compartments. DNA replication, transcription, protein synthesis, central metabolism and respiration all take place in the same environment. Complex biochemical processes may nonetheless be spatially organized in the cell. Transcription of DNA into mRNA and translation of the mRNA into protein are coupled processes. This means that polysomes are linked to the DNA, via the enzyme RNA polymerase (Fig. 1.5). The cytoplasmic membrane not only contains numerous metabolite transport systems, but it is the site of intense enzymatic activity as well. Like eukaryotic cells, bacteria possess efflux systems that allow them to expel unwanted substances from the cytoplasm into the environment. Gram-positive bacteria express many important enzymes and ligands in their cell wall. It is estimated that *Listeria monocytogenes* has 42 different cell wall anchored proteins.

The genetic information is usually stored in a single circular chromosome. Bacterial chromosomes vary considerably in size. The *Haemophilus influenzae* chromosome, the first completely sequenced genome of a cellular life form, is 1.83 million base pairs (Mbp) long and encodes 1703 putative proteins.[13] The chromosome of laboratory strains of *E. coli* K12 is approximately 2.5 times bigger (5 Mbp), though still rather small if compared with the 30 Mbp *Bacillus megaterium* genome that is more than 500 times the length of the cell (Fig. 1.6). A few organisms such as *Vibrio cholera* and *Borrelia* spp. have fragmented genomes, and the *Borrelia* genomes are encoded on linear DNA. The advantage to the organisms of such an arrangement is not known.

The bacterial chromosome codes for polypeptides and stable RNA molecules such as transfer RNA and ribosomal RNA molecules. *E. coli* probably contains well over 1500 different polypeptides with a variety of functions, including maintenance of membrane structure; transport; respiration; degradation of nutrients; synthesis of amino acids, sugars, nucleotides, lipids and vitamins; and production of polymers such as DNA, RNA, proteins and polysaccharides. Mobile genetic elements, such as plasmids, bacteriophages and transposable elements, are important sources of genetic variation. They supply genes that are not essential for bacterial growth but may offer a selective advantage under specific conditions. Virulence factors and antibiotic resistance elements are frequently associated with these mobile DNA structures.

Comparison of the genome sequences of the harmless laboratory strains of *E. coli* shows that naturally occurring *E. coli* isolates can differ by up to 1 Mb, ranging from approximately 4.5 to 5.5 Mb. Thus the commensal *E. coli* K12 has a genome of 4.64 Mb, while the human pathogen *E. coli* O157:H7 has a genome of 5.53 Mb. Several uropathogenic *E. coli* (UPEC) have been sequenced and have genomes varying from 4.94 to 5.23 Mb. The differences in genome sizes are largely due to insertions or deletions of large chromosomal regions, referred to as pathogenicity islands. Genomic islands in UPEC that are not found in commensal *E. coli* account for more than 10% of the genome, emphasizing the importance of lateral gene transfer in the evolution of pathogens. The overall gene order, except for the insertions, remains the same in all *E. coli* and is remarkably similar to the gene order in other Enterobacteriaceae such as *Salmonella enterica*.

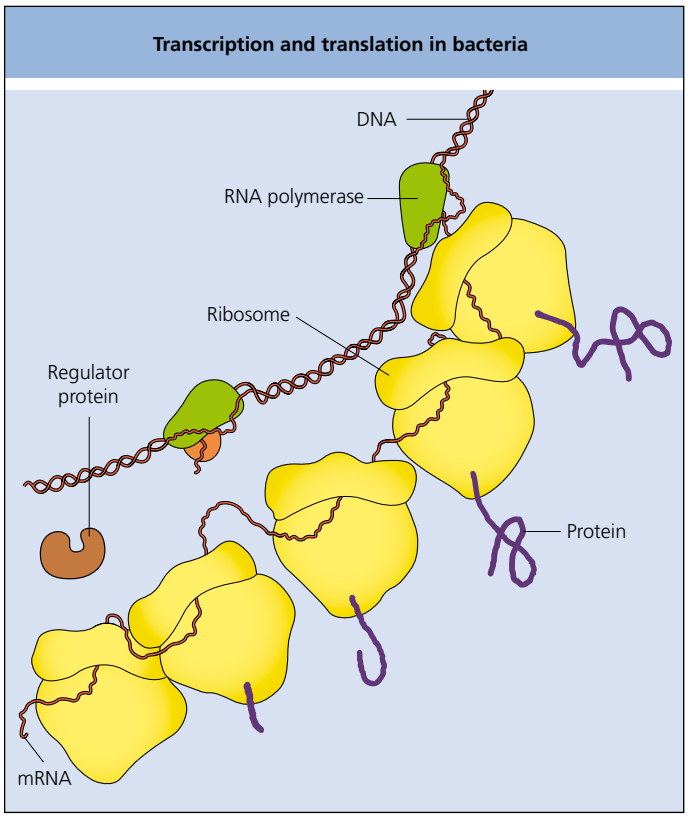


Fig. 1.5 Transcription and translation in bacteria (*Escherichia coli*). (Courtesy of Menno Kok & Jean-Claude Pechere.)

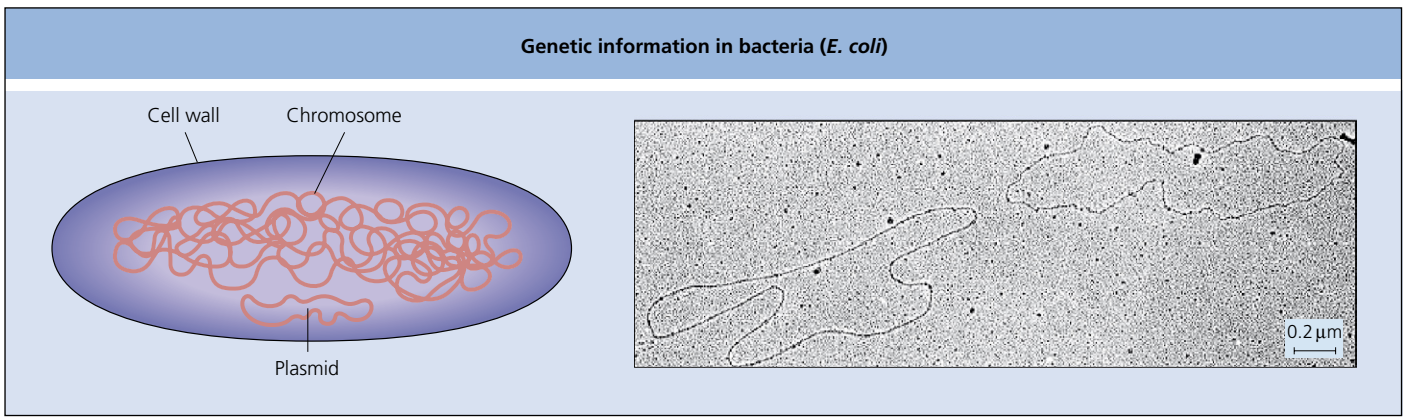


Fig. 1.6 Genetic information in bacteria. This example is *Escherichia coli*. Additional genetic information may be supplied by extrachromosomal elements such as plasmids or bacteriophages. Bacteria may carry a variety of these 'mobile genetic elements', which may transfer readily from one cell to another. The electron micrograph shows a 8.65 kb *E. coli* plasmid that confers sulfonamide and streptomycin resistance (left) and a single-stranded derivative of the plasmid (right). (Courtesy of Menno Kok & Jean-Claude Pechere.)

Transcription and translation in bacteria

Gene expression is usually regulated at the level of transcription initiation by regulator proteins and occasionally by small RNA molecules, which interact with the 'promoter DNA' and with the enzyme RNA polymerase (see Fig. 1.5). The promoter is the site where RNA polymerase opens ('melts') the dsDNA to synthesize an RNA copy of one of the two DNA strands. A sigma factor transiently interacts with the polymerase when it binds the promoter DNA and determines the nucleotide sequence specificity of the enzyme. Bacterial cells produce multiple sigma factors, each controlling the expression of a set of genes, and each expressed under different environmental conditions.

Three types of RNA are produced: regulatory RNA, 'stable' RNA and messenger RNA (mRNA). Stable RNAs include the transfer RNA molecules, which position the amino acids on the ribosomes during protein synthesis and are important structural components of the ribosomes. Messenger RNA molecules are generally quite unstable but are protected from premature degradation by ribosomes, the protein synthesis machines.[14] Regulatory RNAs, such as small RNAs (sRNA) in two component systems, may function in a fashion similar to microRNAs in eukaryotes.[15] Transcription and translation are coupled in bacteria; ribosomes bind the mRNA as soon as it 'leaves' RNA polymerase and start protein synthesis by coupling the initiator amino acid (formyl-methionine) to the second amino acid in the coding sequence and uncoupling it from the tRNA molecule. As mRNA elongation proceeds, more ribosomes bind to the mRNA to form a 'polysome'. The polypeptides that are produced by the ribosomes fold either spontaneously or with the help of molecular chaperones into their native structures. Bacterial mRNAs generally encode more than one protein. The bacterial protein synthesis machinery is an important target for antibiotics.

Motility

Many bacterial species can detect very small variations in concentrations of either valuable or harmful substances in the surrounding environment, guiding movement in a process called chemotaxis.[16] Flagella are the effectors of chemotaxis (Fig. 1.7). By changing the direction of flagellar rotation, micro-organisms swim towards sites favorable to survival and growth and away from noxious stimuli. Amino acids and sugars are powerful chemoattractants. Although many pathogenic species are flagellated, a role for motility in virulence has not been established in many cases.

PATHOGENESIS OF INFECTIOUS DISEASE

The key microbial factors involved in the onset and spread of microbial infection can be identified by carefully analyzing the interaction of the micro-organism with its host (Table 1.4). Insight into the intimate relationship between host and pathogen will help us find the answers to the all-important questions: how can we eliminate the cause of disease and how can we reduce its harmful effects on the human body?

One of the major advances in pathogenic microbiology has been the use of molecular techniques to make targeted mutations in organisms. These mutants can then be tested in appropriate animal or tissue culture models to determine if the loss of the gene affects the virulence of the pathogen without affecting its ability to grow *in vitro* in standard media. Genes that are identified as 'virulence' genes are sometimes considered to be 'accessory' genes because they are not required for replication outside the host. A virulence gene can also be cloned into a genetically related nonpathogenic microbe and tested for its ability to confer a new property in that organism such as adhesion or hemolysis.

Virulence factors can be thought of as falling into one of two functional categories, though there can be overlap in the categories. There are purely defensive functions that help the organism to escape the host's innate immune response. Two examples of this are the polysaccharide capsule made by *Streptococcus pneumoniae* and the golden pigment made by *Staphylococcus aureus*. The former prevents complement from effectively opsonizing the bacteria for ingestion and destruction by neutrophils, while the carotenoid pigment

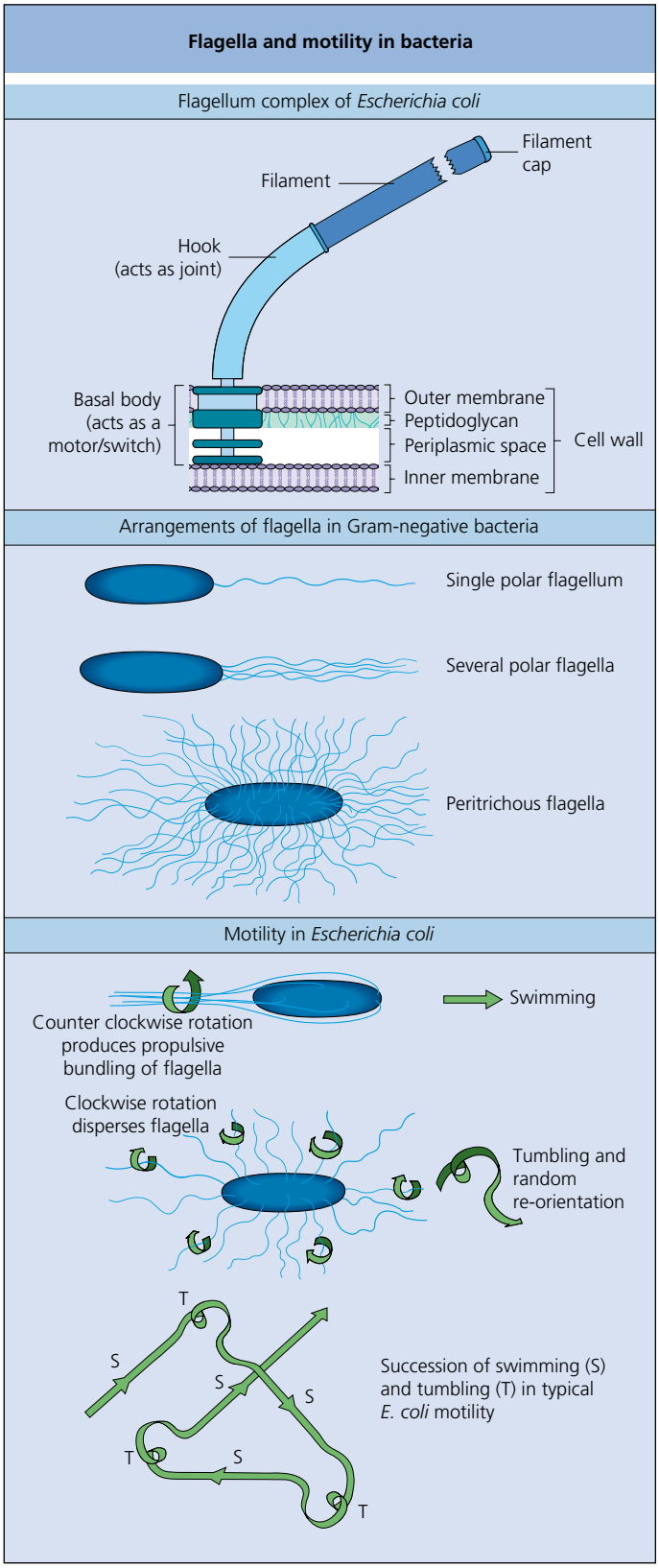


Fig. 1.7 Flagella and motility in bacteria. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Table 1.4 Important steps in microbial pathogenesis

- Encounter
- Attachment to host cells
- Local or general spread in the body (invasion)
- Cell and tissue damage
- Evasion of host defenses
- Shedding from the body

(staphyloxanthin) that gives *Staph. aureus* its name is an antioxidant that helps the bacteria to survive the oxidative damage inflicted by the respiratory burst of phagocytes. In contrast, many exotoxins (e.g. cholera and diphtheria toxins) secreted by the bacteria actively inflict damage on the host and so can be thought of as offensive weapons. Lipopolysaccharides (LPS) serve both functions for the organism. The polysaccharide chains divert the membrane attack component of complement from the inner membrane, making Gram-negative bacilli resistant to the bactericidal action of complement (defensive), while the lipid A end can stimulate exuberant and damaging inflammation by binding to the TLR4/MD-2/CD14 complex.

Because bacteria do not constitutively produce virulence factors that are not necessary for their growth, they transcriptionally regulate expression of virulence genes. Since expression of these regulated virulence genes (or 'accessory genes') may carry a large metabolic cost, organisms regulate their expression in response to environmental signals. Mutation of the regulators often affects the expression of many genes, both positively and negatively, and regulatory mutants are more impaired and less virulent than most single gene mutants. Examples of this include the phoP/Q regulon in *Salmonella enterica* and the agr regulon in *Staph. aureus*. Some regulators sense environmental conditions such as pH or magnesium concentration and others sense bacterial density via quorum sensing.

Viruses face problems similar to bacteria in the host, but must solve them using a limited genetic repertoire. Analogous to bacterial virulence factors, viral genes required for replication and/or pathogenesis in the host that are dispensable for replication in tissue culture are termed accessory genes. For example, simian immunodeficiency virus (SIV) strains lacking the *nef* gene replicate well in certain cell lines but are much less capable of producing disease.[17] Expression of both SIV and HIV *nef* both increases viral infectivity and assists in evasion of adaptive host immunity by downregulation of major histocompatibility complex (MHC) class I, needed for presentation of antigen to allow recognition of infected cells by cytotoxic lymphocytes. The HIV *vif* gene inactivates an innate restrictive factor, APOBEC3G, which otherwise renders progeny virus uninfectious.[18]

Lifestyles and pathogenesis

Each pathogen has its own infection strategy. In the following sections we shall examine the lifestyles of some pathogenic species.

Contamination

In the developed areas of the world, the majority of human infections are caused by pathogens that either belong to the normal microflora of the host (so-called endogenous infections), though there are many exceptions. Infections caused by exogenous micro-organisms have steadily declined over the past century. In contrast, exogenous infections are still prevalent in poorer areas.[19]

Endogenous infections and normal microbial flora of the human host

The fetus in utero is normally sterile but immediately after birth it starts acquiring its indigenous microflora, which will quickly outnumber its own cell content; a normal adult carries more than 10^{14} bacteria, which represents roughly 10 bacteria for each eukaryotic cell. In addition to bacteria, we provide permanent or transient hospitality to an estimated 150 viral species, including numerous remnants of 'endogenous' retroviruses that are inherited in our DNA,[20] to a few fungi, some protozoa and occasionally to worms. The indigenous flora, or 'normal flora' that consists of many species of bacteria and one fungus (*Candida albicans*), is found in any part of the body exposed to the outside environment – the alimentary tract from the mouth to the anus, nose and the oropharynx to the epiglottis, the anterior part of the urethra and the vagina, and the skin (Fig. 1.8). The human microbial population is especially dense in the large intestine; it has been estimated that each gram of stool specimen contains about 10^{12} bacteria. The normal flora is well adapted to its niche and may multiply rapidly under favorable nutritional conditions such as those found in the colon. Although the host's age and physical condition, and especially antibiotic treatment, may induce important variations, the microbial population of the gastrointestinal tract seems to be stable, consisting of more than 99% of obligate anaerobic species. The fecal flora is much more diverse in vegetarians than in omnivores or carnivores, probably reflecting the difficulty of digesting complex carbohydrates found in plants. Facultative anaerobes such as *E. coli*, which are frequently used as markers for environmental pollution with human feces, represent less than 1% of the normal flora.

Transient micro-organisms, ingested with food or water, will normally pass through the proximal small intestine because of the high flow rate and low gastric pH, without being able to penetrate the mucous gel that overlays the intestinal epithelium or to adhere to the epithelial surface. Population levels of the different areas of the gastrointestinal tract are controlled mainly at the level of metabolic competition, the normal flora being well adapted to the low oxidation reduction potentials and tightly adherent to the mucosal epithelium. Pathogens that use the gastrointestinal tract as a portal of entry must find ways of dealing with the fierce microbial competition in the colon, or they target the less densely populated small intestine. Small intestinal pathogens have specific adhesions that allow them to remain attached to epithelial cells or to invade those cells.

The skin is much less densely populated by the indigenous flora. In comparison with the gastrointestinal tract, it supplies a considerably less stable microenvironment and one that is often devoid of water. Nevertheless, there are bacteria within skin appendages in all areas of the skin. Although intact skin is impermeable to bacteria, a number of parasites, among them *Schistosoma mansoni*, which poses a major health threat in developing countries, can penetrate the intact human skin. Skin disruptions due to lacerations or insect bites may allow entry of pathogenic microbes into the body.

The large majority of micro-organisms in the human flora reside on the body surface without creating any damage. This peaceful cohabitation can be called a symbiotic ('both sides win') relationship. Some bacteria find shelter and food in the intestine and, in turn, supply vitamins or digest cellulose. We are just beginning to understand the roles that normal flora play in human nutrition, in maintaining oral tolerance and in developing our innate immune system. If the micro-organisms, rather than the host, derive benefit from the association, those inhabitants of our body are called commensals. True commensals do not invade the host and, therefore, do not elicit an immune response. Parasitism constitutes a third category where the micro-organisms, after invading the host, cause an infection. Some have suggested that chronic infection with highly prevalent viruses, including herpesviruses, may play a protective role against bacterial infection by boosting innate immunity, suggesting a complex, three-way symbiotic relationship.[21]

The separation between parasitism, commensalism and symbiosis is not always clearly defined and the condition of the host may make a big difference. Some micro-organisms, referred to as opportunistic pathogens, are commensals in the majority of people but can cause disease in an immunocompromised host. For instance, *C. albicans* is part of the normal oral flora, but in the absence of adequate numbers of CD4 T cells, as occurs in AIDS, the yeast can proliferate and cause thrush and esophagitis. Similarly, virtually all individuals chronically

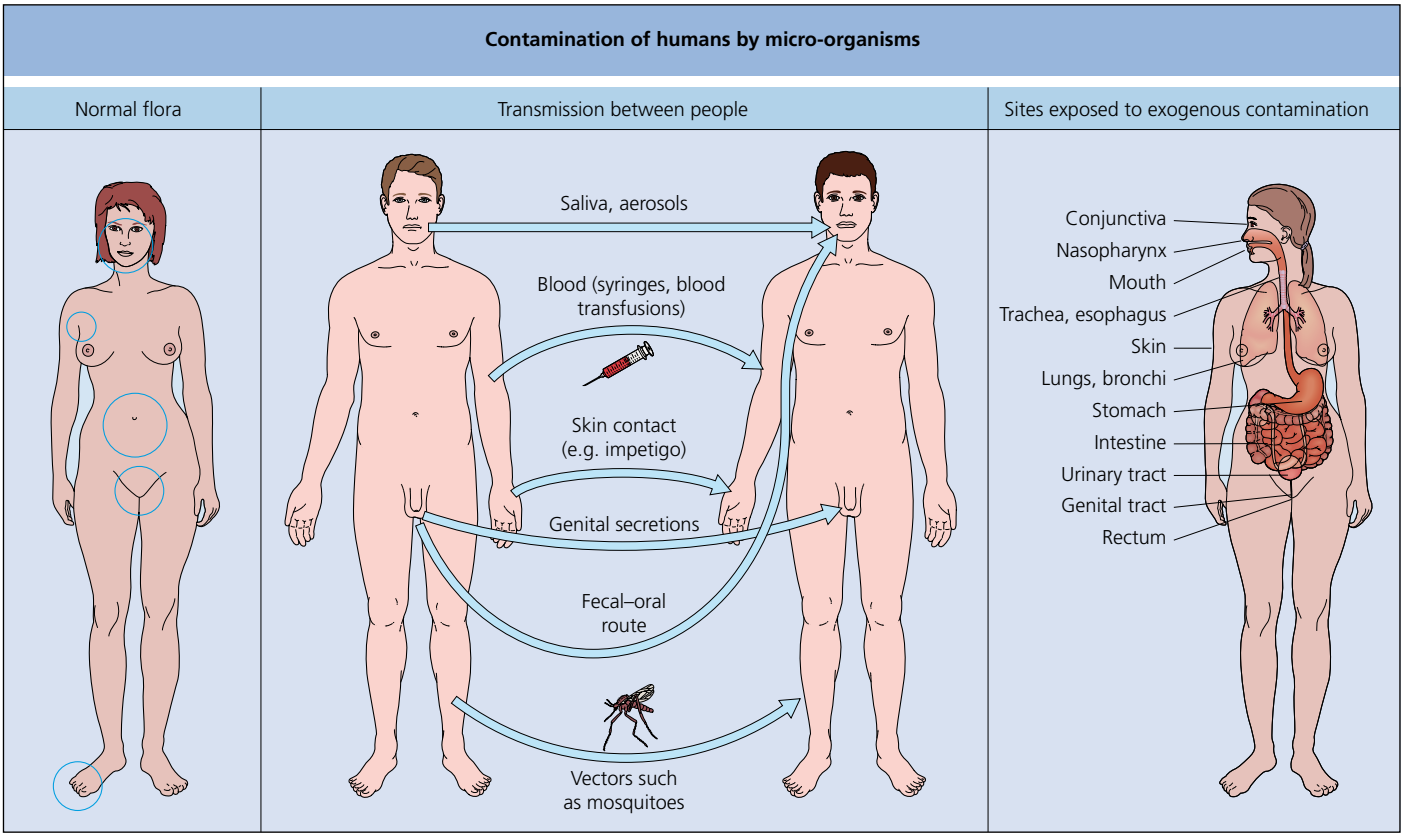


Fig. 1.8 Contamination of humans by micro-organisms. Many parts of the body are colonized by normal flora, which can be the source of endogenous infection. Large numbers of micro-organisms are found in moist areas of the skin (e.g. the groin, between the toes), the upper respiratory tract, the digestive tract (e.g. the mouth, the nasopharynx), the ileum and large intestine, the anterior parts of the urethra, and the vagina. Other routes are interhuman transmission of infections and exposure to exogenous contamination. (Courtesy of Menno Kok & Jean-Claude Pechere.)

infected with human cytomegalovirus (CMV) are asymptomatic, but CMV can cause serious diseases such as colitis and pneumonia when the immune system is suppressed.

The host and its indigenous microflora maintain a delicately balanced relationship that, when disrupted, may lead to the development of infectious disease.

An inevitable consequence of antibiotic treatment is the elimination of susceptible bacteria, which are quickly replaced by antibiotic-resistant species. This phenomenon can cause diseases such as mucosal candidiasis, pseudomembranous colitis or enterococcal superinfection. Even a short course of antibiotics can cause a large change in the composition of the fecal flora.

Probiotics (live micro-organisms) may help to restore the natural flora after antibiotic use, but their usefulness is still not fully established. For example, *Saccharomyces boulardii* may be used to prevent relapses of colitis caused by *Clostridium difficile,* but there are no convincing studies showing efficacy.

Exogenous infections

Exogenous infections occur after direct contamination from microbial populations in the environment.

Humans are continuously in intimate contact with the large exogenous microbial populations in the air, soil and water, which may harbor highly pathogenic bacteria such as *Clostridium tetani* and *Bacillus anthracis*. Important pathogenic species, such as *Salmonella enterica, Staph. aureus, Clostridium perfringens* and *Clostridium botulinum*, may be present in our food and cause food poisoning or gastroenteritis.

Live animals represent another important source of exogenous micro-organisms. Infectious diseases of animals that may be transmitted to humans (called zoonoses) include cat-scratch fever, brucellosis, tularemia, toxoplasmosis and rabies. In addition, microbial pathogens can be transmitted from animals to humans by insect vectors such as flies, mosquitoes and ticks. Plague and Lyme disease are examples of vector-borne zoonotic bacterial infection. Many viruses, such as dengue virus, which is transmitted by Aedes mosquitoes, are transmitted by insect vectors. The Sin Nombre hantavirus, which produces hantavirus pulmonary syndrome, is acquired from rodents by aerosolization of their dried, infected urine. Many protozoan pathogens are transmitted by insect bites, malaria being the most important.

The most important sources of exogenous infections are probably humans themselves (see Fig. 1.8). Well-known examples of human-to-human transmission include AIDS and other sexually transmitted diseases, airborne infections such as varicella, rubella, measles and tuberculosis, and fecal–oral infections such as shigellosis and typhoid fever. Vertical transmission of infections to the fetus or newborn is uncommon but they often have devastating effects. They include toxoplasmosis, CMV, rubella, HIV, listeriosis and syphilis. In contrast, vertical transmission of hepatitis B virus (HBV) occurs at or after parturition, and the infants have a high likelihood of becoming asymptomatically but chronically infected. This is common in many populations in South East Asia where HBV is endemic. Cross-infection in hospitals poses enormous problems, especially in intensive care units, but these infections are usually transmitted on fomites or inadvertently on the hands of hospital personnel rather than by direct contact or by droplets.

Several regions of the body may be exposed to exogenous contamination (see Fig. 1.8). Healthy people may be carriers if they harbor and excrete potentially disease-producing micro-organisms. For instance, people recovering from typhoid fever may retain *Salmonella typhi* in the gallbladder and continue to excrete the pathogen in the feces long after recovery from the disease. These people are chronic carriers, even

though they have recovered from the illness themselves. Certain bacterial respiratory pathogens have no environmental or animal hosts, and are passed by droplets from person to person. These include *Strep. pyogenes, Strep. pneumoniae* and *Neisseria meningitidis*. If newly colonized individuals do not have protective antibodies they are liable to develop symptomatic infections. Other than pre-existing immunity, why some people become ill after they acquire these organisms and others remain asymptomatic is not well understood.

A small number of exogenous pathogens are airborne and establish infection by direct interactions with alveolar macrophages or mucosal dendritic cells. For this to happen the particles must be of a certain size; particles larger than 4 microns in diameter will not reach the terminal airways and very small particles will be trapped in the nasopharynx. Alveolar macrophages are inherently downregulated for inflammatory responses, which is probably necessary to prevent lung damage from the many encounters with particles in the air. However, this makes them ill equipped to kill organisms that they may ingest. *Mycobacterium tuberculosis*, the primary pathogenic fungi such as *Histoplasma capsulatum, Paracoccidioides braziliensis* and *Coccidioides immitis*, and the environmental bacterium *Legionella pneumophila* are examples of airborne pathogens.

Exogenous infections, predominant in the past, have dramatically declined in the developed world thanks to improved hygiene, vaccination programs and infection control programs. They are, however, still prevalent in areas with limited resources. Pneumococcal pneumonia, diarrheal diseases from contaminated food and water, malaria, measles, AIDS and tuberculosis are the main causes of mortality in developing countries, other than malnutrition and trauma. In the 1990s there was a large diphtheria epidemic in Russia as the result of the collapse of the public health infrastructure, demonstrating that pathogenic microbes are still in the environment and can become epidemic even in technologically advanced countries if we relax our efforts to contain them.

The infection process

Three stages in the infection process may be functionally distinguished:

- attachment of the micro-organism to the target cell(s) and, for intracellular pathogens, entry into the host cell;
- development of the infection, local multiplication of the pathogen and spread of the micro-organism to distant sites; and
- shedding of the organism and transfer to a new host.

Attachment to host cells

Only a few pathogens have the capacity to penetrate our body directly through the skin. Examples include the cercariae of various schistosome species, which can invade the skin with the help of their glandular secretions. Many other pathogens enter the body after an insect bite (e.g. *Simulium* blackfly bite for *Onchocercus volvulus*, anopheles mosquito bite for malaria) or iatrogenically from intramuscular or intravenous injection of contaminated medications or blood. This can transmit various blood-borne pathogens such as HBV, hepatitis C virus (HCV), HIV, West Nile virus, syphilis and malaria.

Although 'free' micro-organisms exist in the body (for instance, in the lumen of the intestine or in the saliva), most members of the human flora need to be attached to a cellular surface to avoid being swept away by biologic fluxes such as swallowing or the passage of the alimentary bolus. For many microbial and viral pathogens, adherence to the epithelial surface of the respiratory, digestive or reproductive mucosa is a compulsory step in pathogenesis.

Adherence

The approach of micro-organisms to an epithelial surface is guided by a balance between attractive and repulsive forces. Eventually, multiple high-affinity contacts between the microbe or virion and the cellular surface may establish a virtually irreversible association between the two. Even for viruses, attachment may involve multiple different mechanisms. Such contacts may involve nonspecific interactions, such as those between exposed hydrophobic structures on the microbial cell envelope and lipophilic areas on the cell membrane. Glycocalyx, made essentially of a mixture of polysaccharides and 'slime', produced in particular by *Staph. epidermidis*, may mediate nonspecific adherence between prokaryotic and eukaryotic cells. Interestingly, carbohydrate capsules on respiratory pathogens appear to interfere with epithelial cell adherence and bacteria that progress from epithelial colonization to invasion and need capsules to survive after invasion, downregulate capsule expression in order to adhere and invade epithelial cells, the first step in their pathogenesis.

Specific adherence involves microbial adhesins on the one side and host cell receptors on the other. Although the interaction between adhesins and cell receptors may be highly specific, this is not always the case. The specificity can be tested by artificially blocking adherence with an excess of purified adhesin or receptor or with antibodies directed against one of these two. The specificity accounts for the early observation that many pathogens distinctively infect certain areas or organs of the body and not others. For instance, *Strep. pneumoniae* causes pneumonia but not urethritis, whereas *Neisseria gonorrhoeae* exhibits the opposite pattern of specificity. The receptors for poliovirus, rhinovirus and HIV are expressed only by specific cell types, restricting virus infection accordingly. Different strains of influenza virus adhere via the hemagglutinin to different sialic acids, and this largely determines not only their host range but also their organ tropism. These and many other examples support the notion that adhesins determine the tropism of microbial pathogens. On the other hand, cell receptors for many organisms are ubiquitous and these organisms have no tissue or even host restriction, possibly because they encode many adhesins. *Salmonella enterica* serovar Typhimurium encodes 12 different fimbriae, but the binding specificity is known for only two.

Ubiquitous receptors

Fibrinogen, fibronectin, collagen and heparin-related polysaccharides are major components of the extracellular matrix (ECM) that coats the mucosal surface of epithelial cells. Members of the integrin family are involved in the interaction between the ECM and the underlying epithelium. A number of components of the ECM are used as receptors for microbial adhesins and viral receptor proteins. *Staph. aureus* has cell wall proteins that recognize nearly all ECM proteins including fibronectin, elastin, von Willebrand factor, vitronectin and collagen.

Attachment to fibronectin is also required for *Staph. aureus* to invade nonphagocytic cells. Fibronectin specifically binds fibronectin-binding factors on the cell envelopes of other bacteria including *Strep. pyogenes, Treponema pallidum, Mycobacterium* spp. and *Orientia tsutsugamushi*, the etiologic agent of scrub typhus; fibrinogen binds groups A, C and G streptococci and a member of the integrin family binds the major invasion factor of *Yersinia pseudotuberculosis*. Their abundance and structural conservation among mammalian species make ECM components ideal targets for bacterial adhesins.

Bacterial adhesins

Close contact between micro-organism and host cell represents an essential step in pathogenesis. It optimizes the interaction of microbial virulence factors with the target cell to allow the pathogen to penetrate or cause local cell damage, or both. Other possible functions of adhesins include modulation of the inflammatory response, adhesin-directed degranulation from mast cells and adhesin-mediated bacterial phagocytosis by neutrophils. Bacteria use two general strategies to attach themselves to host cells: fimbrial and afimbrial adhesion (Fig. 1.9).[22]

Pili and fibrillae

Attachment of bacteria to the plasma membrane can be mediated by filamentous structures protruding from the bacterial surface, called

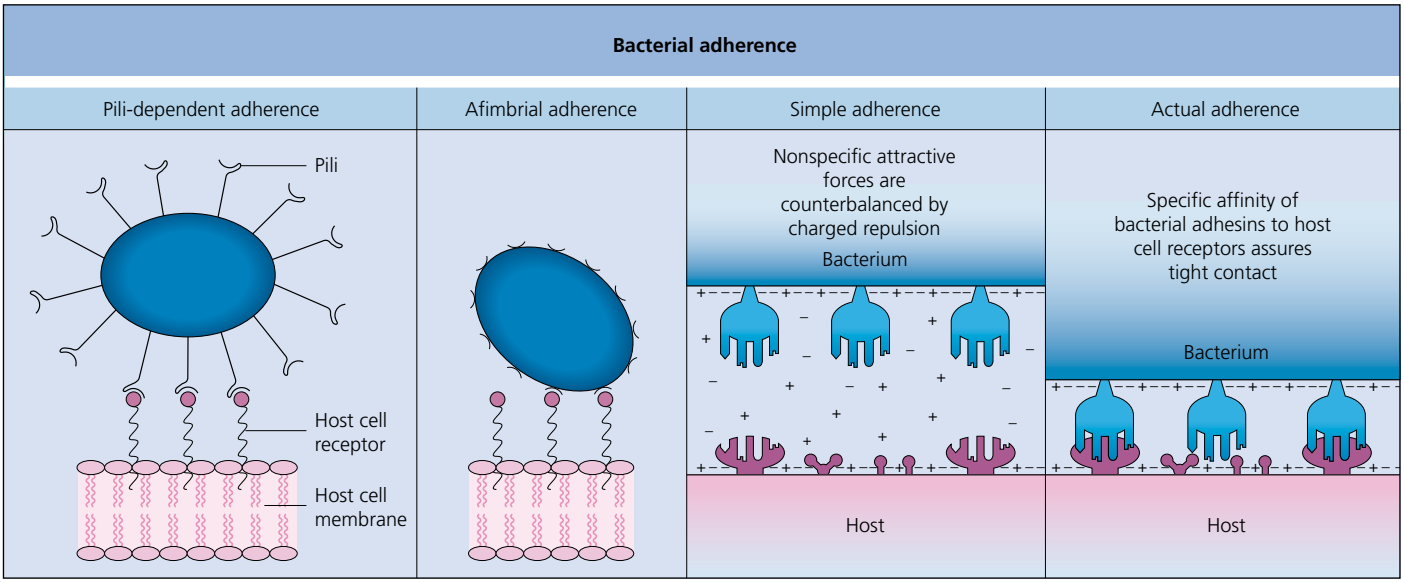


Fig. 1.9 Bacterial adherence. (Courtesy of Menno Kok & Jean-Claude Pechere.)

fimbriae or fibrillae. The classification of these colonization factors is based on morphologic criteria. Fimbriae (or common pili) are rigid hair-like structures with a regular diameter, whereas fibrillae are flexible and have an irregular diameter. These structures are distinct from flagella, which are responsible for bacterial motility (see Fig. 1.7), and sex pili, which are associated with bacterial conjugation.

Twenty different colonization factors have been described for *E. coli*.[23] One of these, the so-called P-pili expressed by uropathogenic *E. coli* strains, mediates adherence of the bacterium to the urinary mucosa to avoid elimination by the urinary flux. P-pili consist of a long and rigid base section attached to an outer membrane scaffold and a short flexible tip (Fig. 1.10).[24] The rigid section is composed of hundreds of pyelonephritis-associated (PapA) pilin subunits arranged in a right-handed helix. The pilus tip is 2 nm in diameter with a 15 nm pitch composed of PapE monomers. The PapG monomer is located at the end of the tip and is the actual adhesin. It recognizes the glycolipid receptor globobiose (α-1–4 linked di-galactose) on the host cell surface.

Afimbrial adhesins

Afimbrial adhesins, such as lectins (carbohydrate-binding proteins), also mediate tight binding between the bacteria and the host cell but, unlike pili, they do not form supramolecular structures. Similar adhesins exist in viruses, fungi and protozoa. Afimbrial binding has been extensively studied in *Strep. pyogenes* (Fig. 1.11). Two surface components are believed to be critical in the colonization of an epithelial surface: lipoteichoic acid and fibronectin-binding protein.

Purified lipoteichoic acid binds to fibronectin and inhibits the binding of *Strep. pyogenes* to oral epithelial cells. The binding properties of *Strep. pyogenes* lipoteichoic acid are confined to the lipid moiety. Similarly, artificially added fibronectin-binding protein inhibits adhesion of *Strep. pyogenes* to epithelial cells even after the streptococci have been depleted of lipoteichoic acid.

The complex surface of this micro-organism also includes the M protein.[26] This protein is a major virulence factor but it does not seem to be involved in adherence to epithelial cells, as was previously assumed. However, the M protein binds fibrinogen in a stoichiometric fashion and exerts an antiphagocytic effect, which may partially explain its role in virulence.

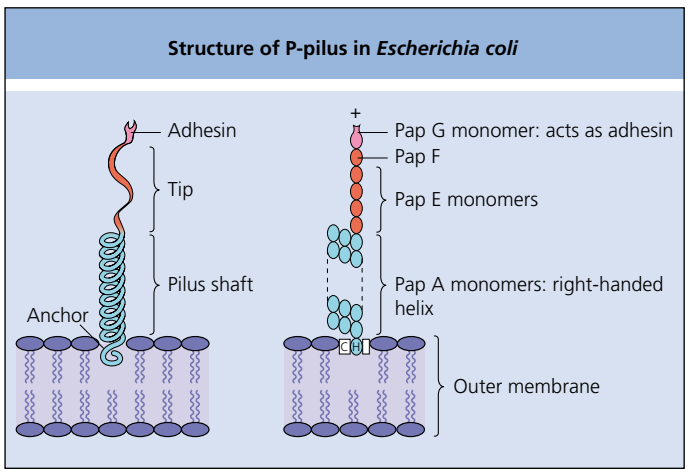


Fig. 1.10 Structure of P-pilus in *Escherichia coli*. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Viral adhesion

Adhesion represents the first in a series of steps that ultimately leads to the delivery of the viral genome to its site of replication. Multiple different viral proteins may be required to mediate attachment, viral fusion and entry into the cell. For example, the HIV gp120 protein first attaches to the CD4 molecule on the cell surface, exposing an area of gp120 that interacts with a seven-loop transmembrane protein co-receptor, finally triggering fusion via a portion of gp41 transmembrane protein. Similarly, different viral proteins in rotavirus interact with membrane carbohydrates, integrins and a heat shock protein to mediate attachment and entry.[27]

For some viruses (typically enveloped viruses, including measles and mumps viruses[28]), attachment proceeds via direct fusion with the cell plasma membrane. These virions have a transmembrane fusion protein that induces contact between the viral and cellular lipid bilayers. Alternatively, attachment may trigger a process of endocytosis, proceeding through clathrin-coated pits, frequently requiring acidification to trigger structural changes in viral proteins that results in escape from the endolysosome into the cytoplasm. This is used by many non-enveloped viruses, including adenovirus, rhinovirus and some other enteroviruses.[29]

Availability of receptors and/or co-receptors on the cell surface determines whether a virus particle will bind. Cell specificity

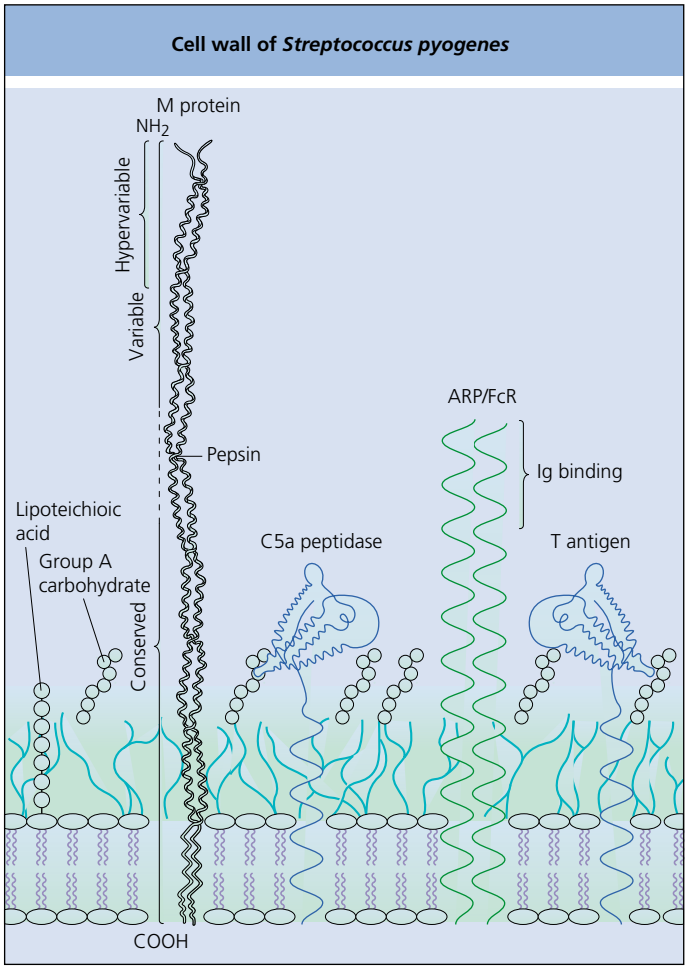


Fig. 1.11 Cell wall of *Streptococcus pyogenes*. The proposed model of the M protein is based on current sequence and structural data. ARP, immunoglobulin A receptor protein; FcR, receptor for the Fc portions of immunoglobulin. Adapted from Kehoe.[25]

('tropism') may be rather relaxed for viruses that use ubiquitous receptors while strongly restricted for viruses requiring two or more cellular receptors. As noted, HIV requires co-expression of CD4 and one of several chemokine receptors for efficient infection, and it principally infects only $CD4^+$ lymphocytes and monocyte/macrophages. In contrast, herpes simplex virus type 1 glycoproteins B and C interact with ubiquitous heparin sulfate present on cell plasma membranes. A second interaction is between glycoprotein D and nectin-1 or herpes virus entry mediator (HVEM) cellular receptors, widely expressed in epithelial cells and some neurons.[30] Herpes simplex virus first infects epithelial cells of the skin and mucosal surfaces, where the initial replication cycles take place, passing into axon termini of neurons. Tropism is not always restricted by surface binding, however. The JC polyoma virus receptor, sialyl (α2–6) Gal, has a wide distribution in human tissues, while virus replication is restricted primarily to oligodendrocytes, urothelial cells and perhaps B lymphocytes.[31]

Viral adherence and invasion can be blocked by neutralizing antibodies, which specifically bind the active site(s) of the adhesin(s). However, many viruses have hidden this region in a protein pocket (or 'canyon'), making it physically inaccessible to potentially neutralizing antibodies, thus escaping humoral immunity. In addition, many viruses produce huge amounts of variation due to relative infidelity of viral polymerases, allowing selection and escape of variants during infection.

INVASION

Invasive and noninvasive micro-organisms

Many micro-organisms, including those of the natural flora, remain at the epithelial surface without invading the underlying tissue (Table 1.5). This type of colonization is usually harmless although it may, in some cases, induce damage to adjacent cells through the production of toxins or elicit a local inflammatory or allergic response. Nonpenetrating micro-organisms include *Corynebacterium diphtheriae*, which cause pharyngitis, *Mycoplasma pneumoniae*, which cause pneumonia, and *Trichomonas vaginalis*, a cause of vaginitis.

Other micro-organisms gain access to deeper tissues only after a physical or chemical injury of the epithelial barrier. *Staph. aureus*, a harmless microbe when on the nasal mucosa, may become a dangerous toxin-producing pathogen once it penetrates the body, or can become locally destructive if it can penetrate beneath the stratified epithelium of the epidermis.

Invasive micro-organisms exhibit the capacity to penetrate the target tissue to which they adhere without the need for local disruption of the protective epithelium. Invasive bacteria have developed the capacity to enter host cells that are not naturally phagocytic. Penetration into these 'nonprofessional' phagocytes is achieved by either engulfment or zippering. *Salmonella enterica* and *Shigella* spp. are examples of bacteria that trigger their own engulfment using type III secretion systems to rearrange the host cell cytoskeleton using enzymes that are injected through the host cell membrane resulting in actin rearrangement so that the bacteria are carried into the cell (Fig. 1.12). The bacteria inject additional enzymes that restore the cytoskeleton to its original shape and restore integrity. In contrast, bacteria such as *Listeria monocytogenes* and *Yersinia pseudotuberculosis* use a 'zipper' mechanism to enter cells that starts with binding to integrins on the cell surface, which leads to cytoskeletal rearrangements. *Listeria* uses a second adhesion factor to enter hepatic cells, attaching to the hepatocyte growth factor receptor, which triggers phosphatidylinositol (PI) 3 kinase activation. Surprisingly, *Listeria* enters all cells in a clathrin-dependent, endocytic manner, except that after clathrin and dynamin are recruited there is cytoskeletal rearrangement necessary for the large particle to enter the cell.

In some cases infection remains confined to the epithelial surface (see Table 1.5), but in others the micro-organism may be transported across the superficial epithelium to be released into subepithelial space. This process is called transcytosis and involves the host cell actin network (see below). After transcytosis, the underlying tissues may be invaded and infected and the infection may eventually spread all over the body (e.g. *N. meningitidis* may cross the pharyngeal epithelium and cause meningitis, and *Salmonella enterica* serovar Typhi may cross the intestinal epithelium and infect the reticuloendothelial system causing typhoid fever). Some pathogens such as *Strep. pyogenes* usually cause disease on an epithelial surface, but they are also capable of invading epithelial cells and causing deep tissue infections. For a more detailed analysis of the mechanisms of invasion, we shall use the example of enteroinvasive pathogens.

Enteroinvasive pathogens and the membranous cell gateway

Acute infectious diarrhea may cause the clinical spectrum ranging from watery diarrhea to dysentery (bloody diarrhea). It occurs when the pathogen invades the intestinal mucosa and causes structural damage to the intestine. The immunologic protection of the intestine is performed by the gut-associated lymphoid tissues, which are separated from the intestinal lumen by epithelium. The follicle-associated epithelium is covered by membranous cells (M cells) that play a prominent role because they are specialized in the transport of antigens. Enteroinvasive viruses, protozoa and bacteria exploit the transport facilities provided by M cells to invade the host. Entry into (and passage through) M cells

Table 1.5 Interaction of micro-organisms with epithelial cells

	Order	Micro-organism	Disease
Generally confined to epithelial surfaces	Bacteria	*Bordetella pertussis*	Pertussis
		Chlamydia trachomatis	Trachoma, urethritis
		Corynebacterium diphtheriae	Diphtheria
		Streptococcus pyogenes	Uncomplicated pharyngitis
		Vibrio cholera	Cholera
		E. coli (EPEC)	Diarrhea
	Viruses	Coronaviruses	Common cold
		Rhinoviruses	Common cold
		Rotaviruses	Diarrhea
	Fungi	*Candida albicans*	Thrush
		Trichophyton spp.	Athlete's foot
	Protozoa	*Giardia lamblia*	Diarrhea
		Trichomonas vaginalis	Vaginitis
Enter through the epithelium	Bacteria	*Shigella* spp.	Bacillary dysentery
		Brucella melitensis	Brucellosis
		Neisseria meningitidis	Meningitis
		Salmonella typhi	Typhoid fever
		Treponema pallidum	Syphilis
		Yersinia pestis	Plague
	Viruses	Measles virus	Measles
		Rubella virus	Rubella
		Varicella	Chickenpox
		Poliovirus	Poliomyelitis
	Fungi	*C. albicans*	Disseminated candidiasis
	Protozoa	*Toxoplasma gondii*	Toxoplasmosis
		Entamoeba histolytica	Liver abscess

by these pathogens is preceded by adherence. While its pathogenicity is uncertain, enteric infection by mammalian reovirus type 1 involves initial entry through M cells mediated by the capsid proteins σ1 and μ1.[32] Infection by poliovirus may proceed by a similar route.[33]

Enteroinvasive bacteria such as *Salmonella*, *Shigella* and *Yersinia* spp. appear to distinguish between different subsets of M cells. Membranous cells produce glycocalyx, which contains a distinctive profile of lectin-binding sites. Diversity in lectin-binding sites between different locations of the gut may account for the tropism of enteric pathogens, such as the preferential colonization of colonic mucosa by *Shigella* spp. rather than *Salmonella* spp., which are more commonly found at the end of the ileum. Following adherence, the interactions with the M cells vary according to the pathogen (Fig. 1.13). Enteroadherent *E. coli* are not internalized and hence are not invasive. *Vibrio cholerae* is taken up and transported by the M cells but rapidly killed thereafter. It is considered to be invasive at the cellular level but not at the clinical level.

The *Salmonella* and *Shigella* spp. genes involved in invasion of the eukaryotic host cell are homologous and have been remarkably well conserved with respect to both the individual coding sequences and their genetic organization (Fig. 1.14).[35] Detailed molecular analyses of virulence factors produced by enteroinvasive *Shigella* spp. have revealed that all virulent species harbor a 220 kb plasmid, of which a 31 kb operon, encoding 32 genes, is both necessary and sufficient for invasion of epithelial cells.[36] The *Salmonella* spp. entry functions are clustered in a 35–40 kb pathogenicity island[37] inserted in the chromosome at centisome 63.[38] Using a needle-like complex,[39] the bacteria translocate a number of effector proteins into the cytosol and the plasma membrane of the target cell.[40] Some of these effector proteins specifically modify the activities of cellular GTPases (see Fig. 1.12), inducing the alterations of the cytoskeleton required for bacterial internalization.

An important difference between the pathogenic lifestyles of these two bacterial species involves the intracellular fate of the bacteria. Once internalized, the bacteria are enclosed by a host cell membrane in an endocytic vesicle, deprived of nutrients. Soon after entry into the epithelial cell, *Shigella* spp. escape from the endosome into the nutritious cytoplasm; however, *Salmonella* spp. have adopted an entirely different strategy. Salmonellae modify the endocytic pathway of the host cell by means of virulence factors encoded largely by pathogenicity islands and, for highly invasive strains, the virulence plasmids, thus avoiding exposure to bactericidal mechanisms of the cell. Although only some of the cellular targets of the translocated bacterial virulence proteins have been identified to date, it is clear that the physiology of the infected cell is profoundly modified to suit bacterial growth and maintenance.

Actin-based intracellular motility of microbial pathogens

Listeria monocytogenes, *Rickettsia* spp., *Shigella* spp. and vaccinia, measles and rabies viruses use active actin modification to move within the cytoplasm of infected cells and to invade neighboring cells. They induce the formation of actin cross-linked filaments, which assemble in characteristic 'comet-like tails' (Fig. 1.15).[41,42] Elongation of the actin filaments generates sufficient force to move the micro-organisms through the cytoplasm at rates of 2–100 mm/min.

The intracellular life cycle of *L. monocytogenes* illustrates this strategy (Fig. 1.16).[43,44] Under natural conditions, *Listeria* first penetrates enterocytes and subsequently spreads through the body to infect a variety of host cells, including endothelial cells, Kupffer cells, hepatocytes, phagocytes and, most importantly, the trophoblasts of the placenta. Entry is facilitated by the products encoded by the internalin (*inl*) family of genes, which seem to confer tropism for different cell types. Once inside the cell, *L. monocytogenes* remains confined to the phagosome for only a short time. Following lysis of the endosomal membrane, it escapes into the cytosol. Membrane lysis is achieved by a production of listeriolysin-O, which attains maximum activity under the acidic conditions of the intravacuolar environment.

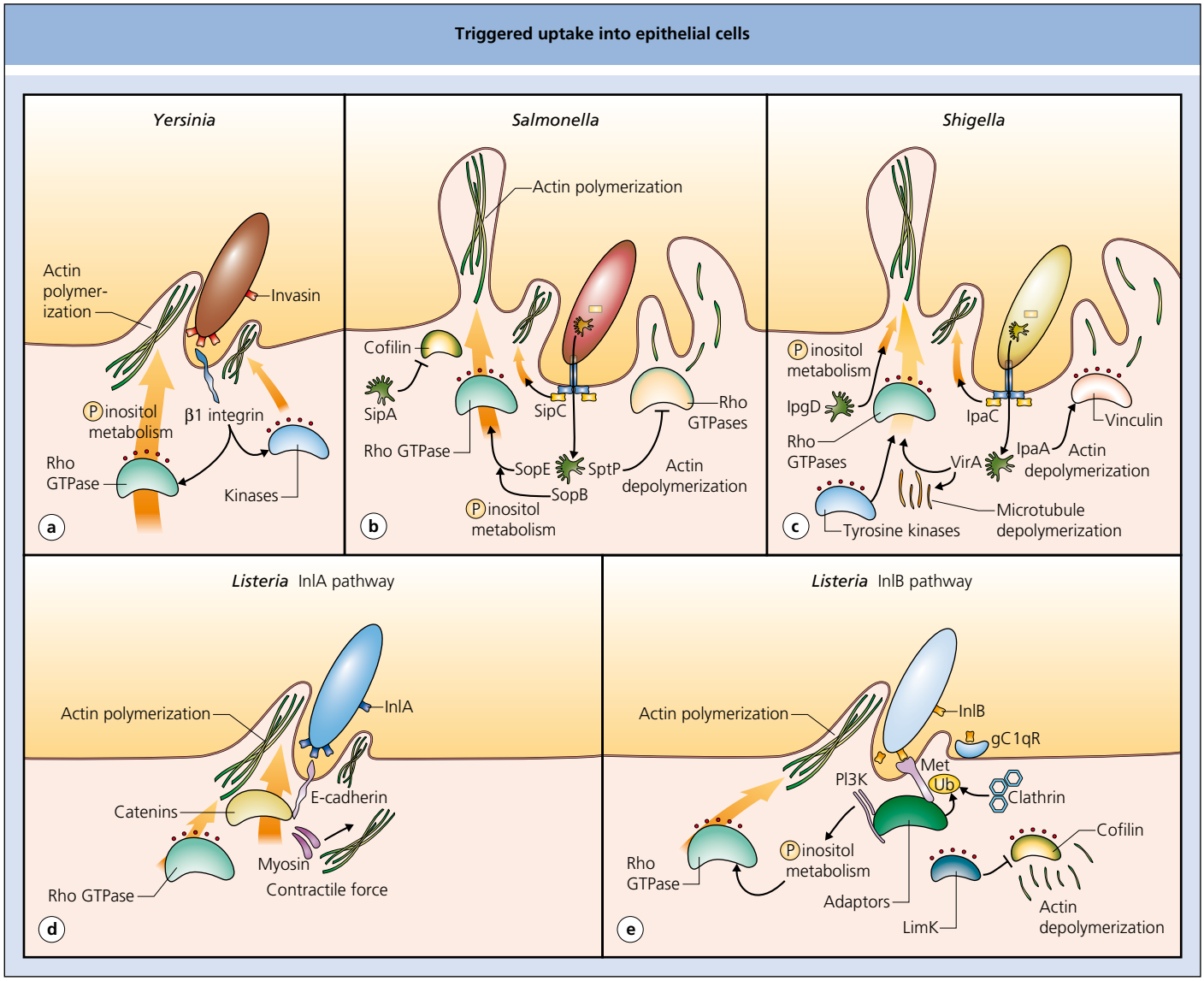


Fig. 1.12 Triggered uptake into epithelial cells. Some invasive bacteria (b and c) express a complex needle-like structure on their cell surface (Type III secretion system) through which they inject proteins that hijack the adjacent cytoskeleton, triggering membrane ruffling that embraces the adjacent bacterium and internalizes it in a vacuole. Others have surface receptors for integrins (a, d and e) which trigger a pinocytic-like response leading to uptake.

Once in the cytosol, the bacteria multiply and migrate towards the plasma membrane by using the actin-based mechanism as described above. Actin polymerization is mediated by the *L. monocytogenes* protein ActA, localized at one end of the bacterium. For spread to neighboring cells, *L. monocytogenes* requires bacterial lecithinase and phospholipase C, which stimulate lysis of the two membranes that separate the bacterium from the cytoplasm of the newly infected cell. Interestingly, most of the virulence genes associated with this process are clustered in a single region of the *L. monocytogenes* chromosome. By spreading in this manner the bacteria are not exposed to human and cellular defenses. However, the intracytoplasmic bacteria stimulate cell innate immune responses via Nod signaling.

Subepithelial invasion and spread through the body

Invasion from the site of infection can only be achieved by micro-organisms that effectively resist or subvert the host defense mechanisms in the subepithelial space, most prominently phagocytosis.

Some organisms take advantage of the normal transport of antigens and are carried by dendritic cells to regional lymph nodes. In the lymph nodes, resident macrophages and polymorphonuclear cells actively fight the invaders. As a result, the first line of lymph nodes is often inflamed. If the invading micro-organism is sufficiently virulent or present in sufficiently large numbers, it may pass into efferent lymphatic vessels to be conducted to the bloodstream. The result is primary bacteremia or viremia.

Some microbes can enter directly into the blood vessels via an injury. A typical example is provided by viridians streptococci, which enter the bloodstream during dental extraction, enabling them to infect an abnormal cardiac valve and produce endocarditis. Insect bites (malaria and arthropod-borne viruses) or damage to the blood vessel wall inflicted during infection with hemorrhagic fever viruses are alternative ways to circumvent the body's first line of defense: the mucosal immune system.

Once in the bloodstream, the micro-organisms may circulate as either an extracellular or an intracellular species. Pathogens have been found in polymorphonuclear cells (*Anaplasma*), lymphocytes (HIV), macrophages (*M. tuberculosis, Histoplasma capsulatum* and CMV) and

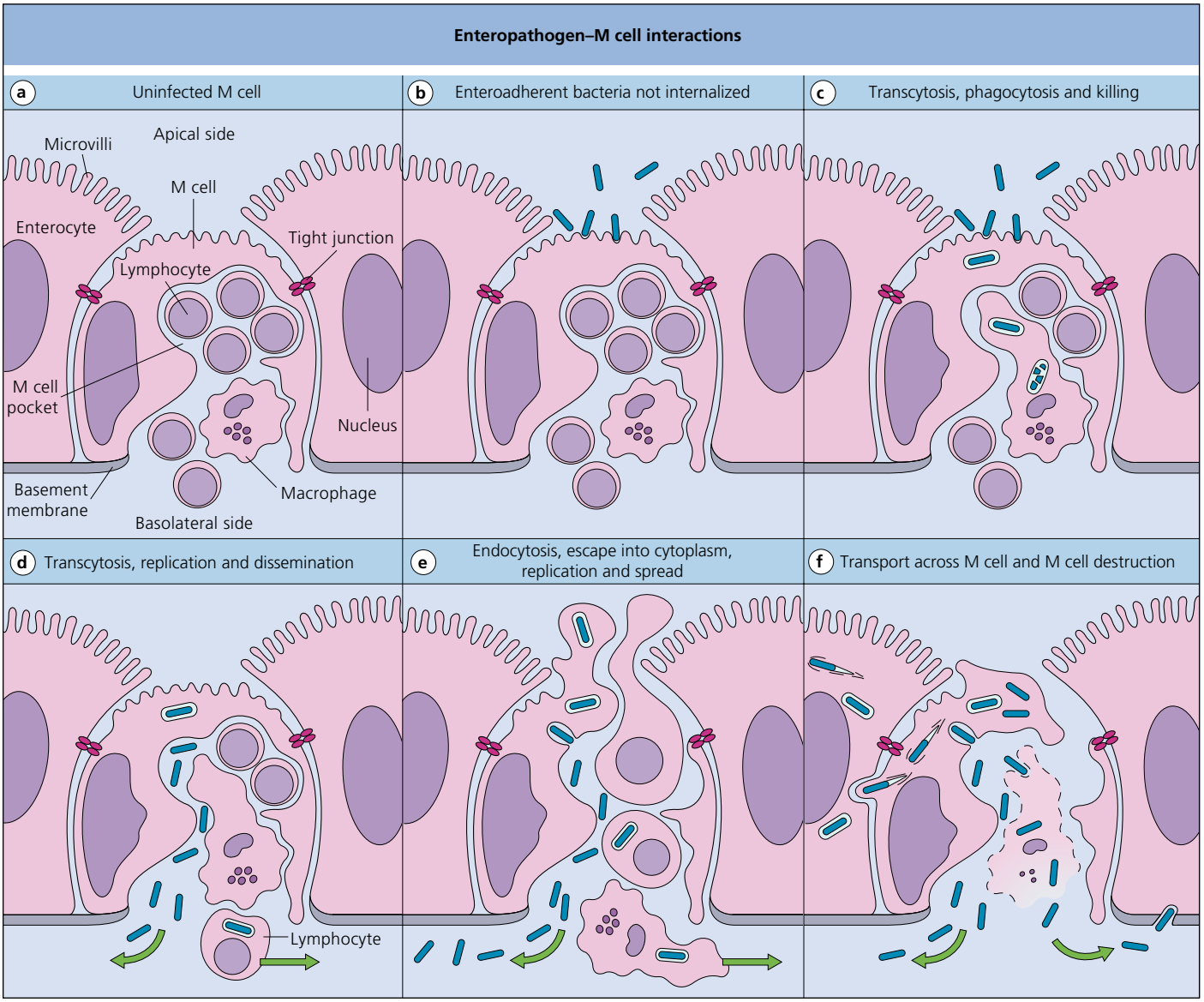


Fig. 1.13 Enteropathogen–M cell interactions. (a) An uninfected M cell, enclosed between two adjacent enterocytes. The basolateral side forms a pocket where lymphocytes and macrophages are located. (b) Enteroadherent *Escherichia coli* forms microcolonies at the M cell surface, but is not internalized. (c) *Vibrio cholerae* undergoes transcytosis but is efficiently phagocytosed in the submucosa. (d) *Campylobacter jejunii* and *Yersinia* spp. undergo transcytosis, replicate in the submucosa and disseminate. (e) *Salmonella* spp. are transported across M cells, leading to destruction of the M cell. (f) *Shigella flexneri* is endocytosed by M cells, escapes into the cytoplasm, replicates, is propelled by actin tails and spreads to adjacent enterocytes. Adapted from Siebers & Finlay.[34]

even in red blood cells (*Plasmodium* spp., *Bartonella bacilliformis*), which provide protection against potent humoral factors in the serum, such as complement.

Infection of distant target organs

Transported by the bloodstream, the invasive micro-organisms can reach distant target organs and create infective metastases throughout the body. Almost any tissue can be reached, but the organs containing abundant capillary and sinusoid networks (e.g. lungs, liver, kidneys, bone marrow) and macrophages that are exposed directly to circulating blood are especially exposed, because blood flows slowly at these sites and transported micro-organisms get the opportunity to adhere and establish an infection. The epiphyses of long bones in children are an important target for certain pathogens such as *Staph. aureus* and *Haemophilus influenzae*. From the target organs, the invaders may produce a secondary bacteremia or viremia, in which microbial counts in the blood are generally higher than during primary infections.

The example of measles virus

Inhaled airborne measles virus recognizes membrane co-factor protein (CD46)[45] and/or the signaling lymphocyte activation molecule (SLAM/CD150)[46] as a receptor on the epithelial surface of the respiratory mucosa. Infection in a nonimmune host proceeds with 2–4 days of limited local replication in the lining of the trachea and bronchi. Pulmonary macrophages carry the virus to the regional lymph nodes, where exponential virus replication causes formation of reticuloendothelial giant cells. Progeny virions enter the bloodstream, causing a primary viremia with spread to the spleen, other lymphatic tissue, the lung, nasopharynx, oral mucosa, thymus, liver, skin and the central nervous system over the next 4–5 days, with secondary replication

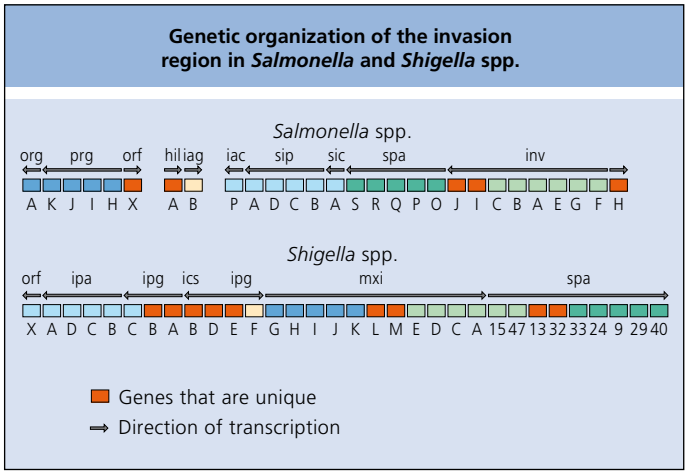


Fig. 1.14 Genetic organization of the invasion region on *Salmonella* and *Shigella* spp. Identical patterns indicate topologically conserved blocks of genes. Each genus has genes that are unique. Despite remarkable genetic similarities, the invasion strategies of the two bacteria are quite different (see Fig. 1.13). Adapted from Galan.[38]

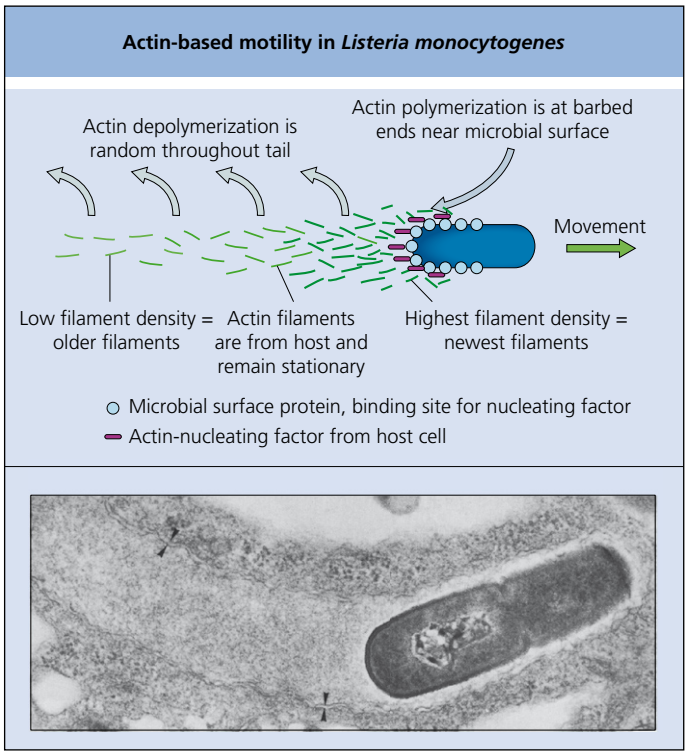


Fig. 1.15 Actin-based motility in *Listeria monocytogenes*. The bacterium moves forwards at the rate of actin-filament growth behind the pathogen. Adapted from Sanders & Theriot.[41] The electron micrograph shows a section of a CaCo-2 cell infected with *Listeria monocytogenes*; the bacterium protrudes into the cytoplasm of an adjacent cell; protrusion is limited by a double membrane (arrowheads).

and increasing viremia. Virus shedding from the nasopharynx begins about 12 days after infection, before symptoms or rash develop, contributing to a secondary attack rate of 80% in susceptible contacts.

These pathogenic steps correspond to different clinical periods. During the 10-day incubation period, infection and primary viremia proceed with no clinical symptoms. Symptoms of fever, malaise, cough and conjunctivitis are concomitant with secondary viremia, followed

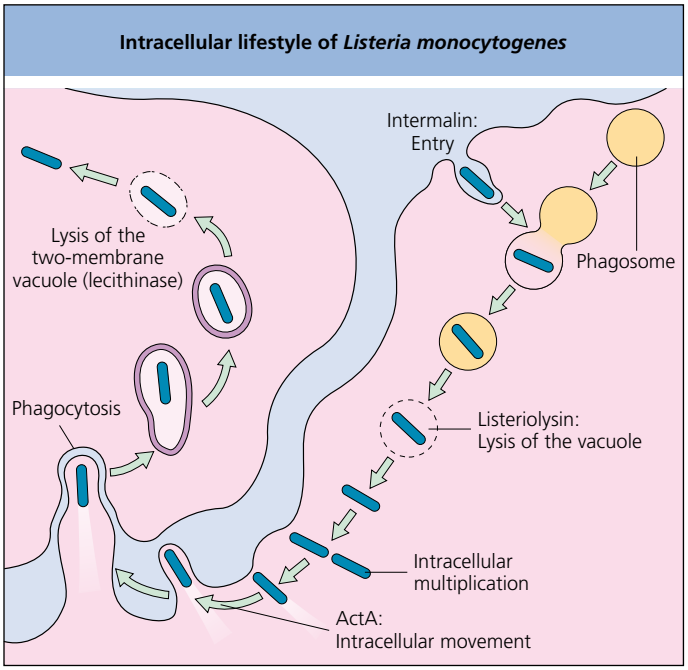


Fig. 1.16 Intracellular life cycle of *Listeria monocytogenes*. (Courtesy of Menno Kok & Jean-Claude Pechere.)

by rash at 12–13 days. The characteristic morbilliform exanthem is associated with a perivascular mononuclear infiltrate, including cytotoxic T cells which migrate to the site of virus infection of dermal endothelial cells and overlying dermis. Virus infection produces epithelial giant cells, but does not directly destroy infected cells in the skin. Leukopenia occurs late in viremia, and immune suppression can be seen from the time of appearance of symptoms until 2–3 weeks after resolution of clinical infection (see Chapter 152).

Serum resistance in *Neisseria gonorrhoeae* and *Salmonella* spp.

Complement is a complex system of circulating proteins that can be activated in three ways:

- by antibodies that interact with C1q;
- by spontaneous hydrolysis of C3 to the enzymatically active C3b that is then stabilized on appropriate surfaces by factors B, D and properdin of the alternative pathway; and
- binding of mannose-binding protein to microbes with subsequent attraction of C1 and mannan-binding lectin-associated serine proteases (MASPs) that proceed to activate C2 and C4.

Complement activation is a major component of the innate immune system, primarily because it can function as an opsonin in people who do not have antibody against the carbohydrates and proteins that form the outer layer of invasive bacteria. Children who are born lacking C3, the central component of all three complement pathways, suffer from repeated bacterial infections and often die in infancy. In contrast, people who are deficient in one of the late complement proteins (C5, 6, 7, 8 or 9) are usually in their teens before they become ill, and the only infections that occur at a higher than expected frequency are disseminated *N. meningitides* and *N. gonorrhoeae*.

In theory, Gram-negative bacteria are susceptible to complement lysis, as the membrane attack unit can insert itself through the outer and inner membranes if C3b binds close enough to the peptidoglycan. However, *Salmonella* and many other Gram-negative bacteria have developed various strategies to prevent complement lysis. These include diverting C3b binding to the ends of the long chain polysaccharide components of LPS that project from the bacterial surface so that the attack component assembles harmlessly at a distance from the inner membrane. Another strategy used by bacteria with short chain

polysaccharides is to use outer membrane proteins to bind host regulatory proteins such as factor H and C4 binding protein to their surface.

Most immunocompetent hosts contracting gonorrhea do not develop a systemic disease because complement can kill bacteria that penetrate through the mucosal barrier. However, the clones of *N. gonorrhoeae* that cause disseminated infections in normal hosts are serum resistant, but often lose that property when passed *in vitro*, as they undergo phase variation. A large number of genes in the *N. gonorrhoeae* genome appear to be phase variable, but which of these is responsible for the invasiveness and complement resistance of disseminated gonococcal infection (DGI) strains is not clear. Another strategy used by pathogenic *Neisseria* to escape complement lysis is stimulation of 'blocking antibodies', which are often IgA (noncomplement binding) that recognize outer membrane proteins and prevent the attachment of bactericidal IgG antibodies and complement to the cell surface.

Cell and tissue damage induced by micro-organisms

Infectious disease is often characterized by cell and tissue damage. Paralysis in poliomyelitis, exanthem in varicella, gastroduodenal ulcers in *Helicobacter pylori* infections and bloody diarrhea in shigellosis all result from damage caused directly or indirectly by micro-organisms. Cell damage can be generated by a variety of different mechanisms (Table 1.6).

Bacterial toxins

Bacteria produce a large diversity of toxins, which have been classified according to their mode of action (Table 1.7, Fig. 1.17). Historically, toxins were defined as soluble substances that alter the normal metabolism of the host cells with deleterious effects on the host. However, as we learn more about the mechanisms of action of exotoxins, the distinction between them and secreted enzymes that play a role in pathogenesis is disappearing. The clostridial exotoxins are good examples of proteases that are exotoxins because they have specific cellular targets within nerve cells. These toxins, which are responsible for tetanus and botulism, are zinc metalloproteases that cleave synaptobrevins or a related protein in the same pathway so that docking and fusion of synaptic vesicles are impaired. The substrate specificity of the proteases and the binding affinity of the heavy chain of the toxins are what determine the different clinical presentations of these diseases. The scalded skin syndrome (SSSS), a blistering skin disorder caused by some strains of *Staph. aureus*, is another example of clinical illness that is the consequence of a secreted protease with a specific target. Cleavage of human desmoglein 1 results in the widespread acantholysis and the flaccid bullous lesions of generalized SSSS.

Cholera toxin ADP ribosylates G_s protein, locking it into the 'on' position, resulting in unregulated activity of adenyl cyclase and high levels of cAMP. The singularity of cholera as an enterotoxin is not due to the specificity of the toxin binding or function but to the location of the pathogen; *V. cholera* is an extracellular mucosal pathogen so only intestinal epithelial cells are exposed to the toxin *in vivo*. Traditionally, exotoxins are said to be excreted toxins. However, some of the so-called exotoxins are actually intracellular and are released into the environment only after cell lysis. The pneumolysin of *Strep. pneumoniae*, for example, is cytoplasmic, the adenylate cyclase of *Bordetella pertussis* is associated with the cytoplasmic membrane and the heat-labile toxin 1 (LT-1) from *E. coli* is periplasmic. The genetic information that encodes bacterial toxins is frequently carried on mobile DNA elements, which may readily pass from one microbial host to another. The toxins associated with diphtheria, cholera, botulism and scarlet fever, as well as Shiga-like toxins in *E. coli*, are encoded by temperate bacteriophages. Genes for LT-1 and methanol-susceptible heat-stable toxin (Sta) of *E. coli* are carried on plasmids.

Toxins deregulate the physiology of the host cell before or during bacterial adhesion and invasion. The bacteria may profit from the induced damage, which compromises the cellular defense against the intruder and release of nutrients from the cytosol. In the case of enteric pathogens, the flux of fluid that characterizes diarrhea makes it more likely that the bacteria will find their way to another host.

The diphtheria toxin as example of an A–B toxin

Diphtheria toxin belongs to the so-called bifunctional A–B toxins (Fig. 1.18). Portion A mediates the enzymatic activity responsible for the toxicity after internalization into the target cell, but cannot penetrate by itself. Portion B is not toxic but binds to a cell receptor localized on the cell surface and mediates the translocation of the A chain into the cytosol. Portion B accounts for the cell specificity of the A–B toxins. The receptor recognized by the B chain of diphtheria toxin is a heparin-binding precursor of epidermal growth factor, an important hormone for growth and differentiation of many different cell types.

Table 1.6 Mechanisms of cell and tissue damage produced by micro-organisms

	Mechanism	Examples
Direct damage by micro-organisms	Production of toxins	See Table 1.7
	Production of enzymes	Proteases, coagulase, DNAses produced by *Staphylococcus aureus*
	Apoptosis	HIV ($CD4^+$ T cells); *Shigella* spp. (macrophages)
	Virus-induced cytopathic effects: Cell enlargement and lysis Formation of syncytium	 Cytomegalovirus Respiratory syncytial virus
	Inclusion bodies: Intracytoplasmic Nuclear	 Rabies Herpesviruses
	Transformation	Human papillomavirus type 16
Damage via the host immune response	Cytotoxic T cells and natural killer lymphocytes	Production of the measles rash, hepatitis A
	Autoimmunity	Acute rheumatic fever
	Immediate hypersensitivity	Rashes associated with helminthic infections
	Cytotoxic hypersensitivity	Cell necrosis induced by hepatitis B
	Immune complexes	Glomerulonephritis in subacute endocarditis
	Delayed type hypersensitivity	Tuberculous granuloma, caseous necrosis

Table 1.7 Examples of bacterial toxins

Toxin type	Example of sources	Toxin	Targets	Mechanisms	Effects
Endotoxin (LPS, lipid A)	Gram-negative bacteria	Endotoxin	Macrophages, neutrophils, B lymphocytes, endothelial cells, plasma components	Activation of target cells via TLR4, complement activation; release of IL-1, TNF, kinins	Fever, septic shock
Membrane-disrupting toxins	*Staphylococcus aureus*	α-Toxin	Many cell types	Formation of pores	Tissue necrosis
	Listeria monocytogenes	Listeriolysin	Many cell types	Formation of pores at acidic pH	Escape from the phagosome
	Clostridium perfringens	Perfringolysin-O	Many cell types	Phospholipase (removes polar head groups from phospholipids)	Gas gangrene
A–B type toxins	*Clostridium tetani*	Tetanospasmin	Synaptic transmission	Inhibits release of inhibitory neurotransmitters	Spastic paralysis
	Clostridium diphtheriae	Diphtheria toxin	Many cell types	ADP ribosylation of EF-2	Myopathy, polyneuropathy
	Vibrio cholerae	Cholera toxin	Intestinal cells	ADP ribosylation of adenylate cyclase, leading to rise cyclic AMP	Profuse watery diarrhea
Superantigen	*Streptococcus pyogenes*	Streptococcal pyogenic exotoxin	T cells, macrophages	T cell stimulation, release of IL-1, IL-2, TNF; possible enhancement of LPS activities	Fever, rash, toxic shock-like syndrome
	Staphylococcus aureus	Toxic shock toxin	T cells, macrophages	Same as streptococcal pyrogenic toxin	Toxic shock syndrome

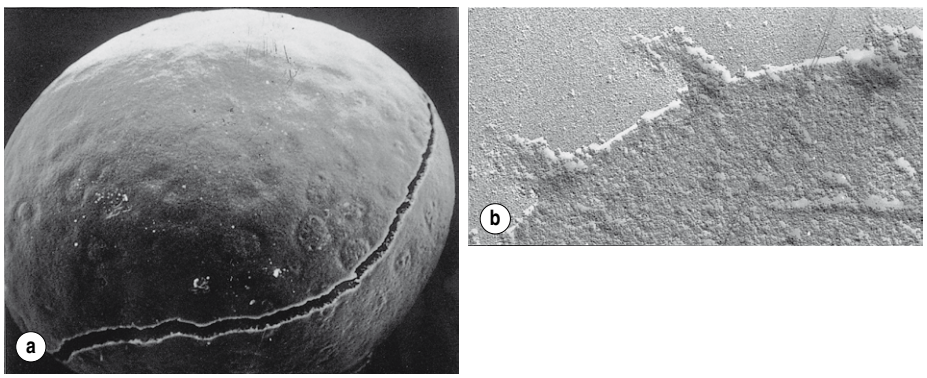


Fig. 1.17 Action of bacterial toxins. (a) *Xenopus* oocyte treated with the cytolytic delta toxin (perfringolysin) of *Clostridium perfringens*. (b) Rabbit erythrocyte exposed to a very small quantity of streptolysin-O, produced by *Streptococcus* A,C,G. Hemoglobin escapes from sites of membrane rupture. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Uptake of diphtheria toxin proceeds via receptor-mediated endocytosis. Acidification of the endocytic vesicle induces a conformational change in the enclosed holotoxin, enabling the A subunit to traverse the membrane and reach its cytoplasmic target. The A subunit of diphtheria toxin catalyzes ADP ribosylation of the elongation factor-2 (EF-2), resulting in its inactivation. The *tox* gene is encoded by a phage and is under the control of the repressor protein DtxR, which forms a complex with iron, DtxR-Fe (Fig. 1.19), binds DNA and represses *tox* expression. Thus diphtheria toxin is only synthesized under low iron conditions, suggesting that it may be produced to stimulate iron release from target cells. Interestingly, the *Pseudomonas aeruginosa* exotoxin A has a very similar structure, but uses a different cell receptor: the α-2 macroglobulin low-density lipoprotein receptor. Like diphtheria toxin, exotoxin enters the cell via receptor-mediated endocytosis but the toxin is released only after passage through the Golgi system.

Hydrolyzing enzymes

Microbial pathogens often secrete hydrolyzing enzymes, such as proteases, hyaluronidases, coagulases and nucleases. As such, these enzymes do not harm the host cells directly and they are therefore not considered to be toxins. However, in the context of an ongoing infection they can facilitate colonization of host tissues by a variety of mechanisms, such as proteolysis of IgA; fluidification of pus; induction of plasma clotting, which may hinder the influx of phagocytes into the focus of infection; and destruction of extracellular DNA nets produced by polymorphonuclear neutrophils (PMNs) as an antimicrobial factor. The release of hydrolytic enzymes by phagocytes damaged by a bacterial toxin may have similar effects.

An example of how one such exoenzyme can nevertheless contribute to the pathogenesis of disease was recently discovered. Host

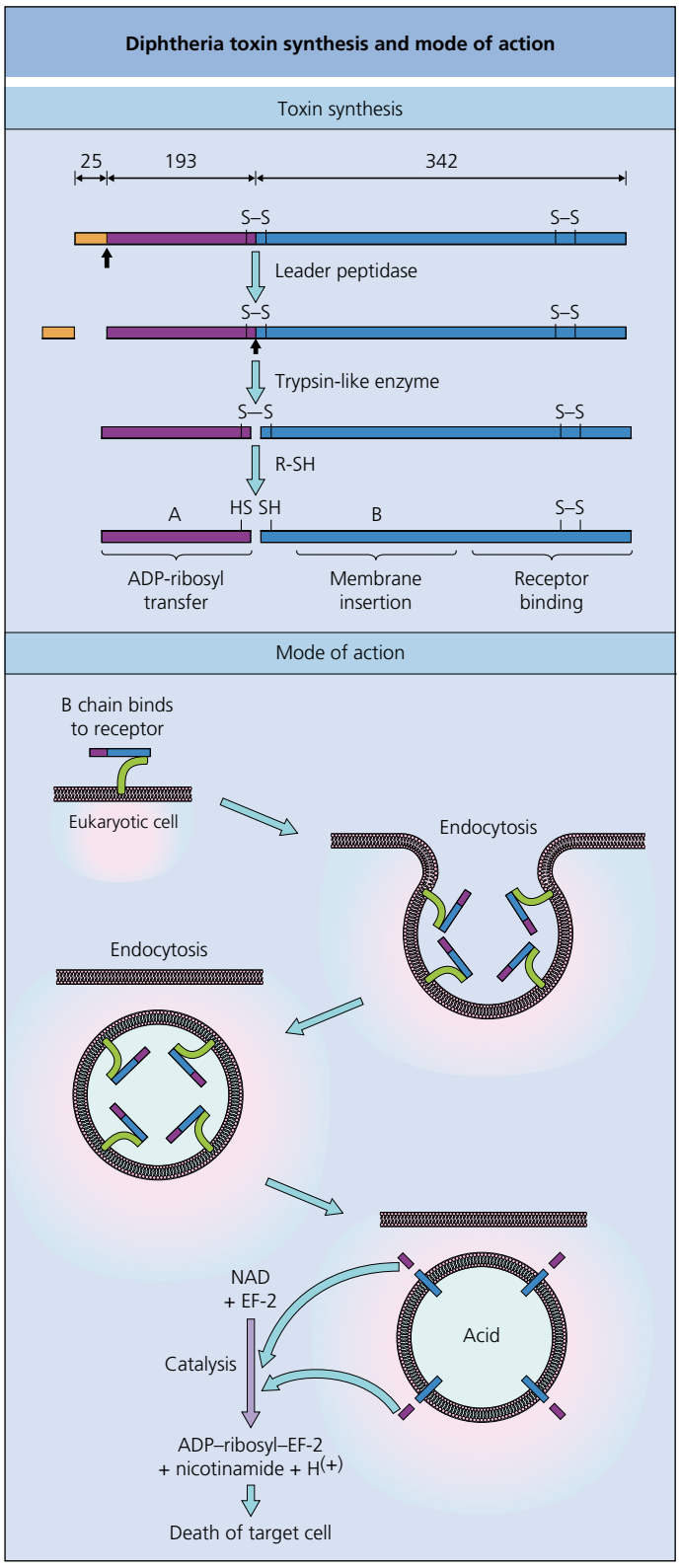


Fig. 1.18 Diphtheria toxin synthesis and mode of action. (Top) The 25-residue leader sequence is cleaved off by the bacterial leader peptidase; the A and B subunits are generated from the precursor protein by a 'trypsin-like enzyme'. Once in the cytoplasm of a targeted eukaryotic cell, the A chain, responsible for ADP-ribosyl transfer, is disconnected from the B chain, responsible for receptor binding and membrane insertion. (Bottom) The B chain binds to a specific receptor on the eukaryotic cell. After endocytosis, acidification in the endosome induces insertion of the B chain into the endosomal membrane and translocation of subunit A into the cytosol, where it catalyzes the ADP ribosylation of EF-2. As a result, protein synthesis is inhibited and the targeted cell dies. (Courtesy of Menno Kok & Jean-Claude Pechere.)

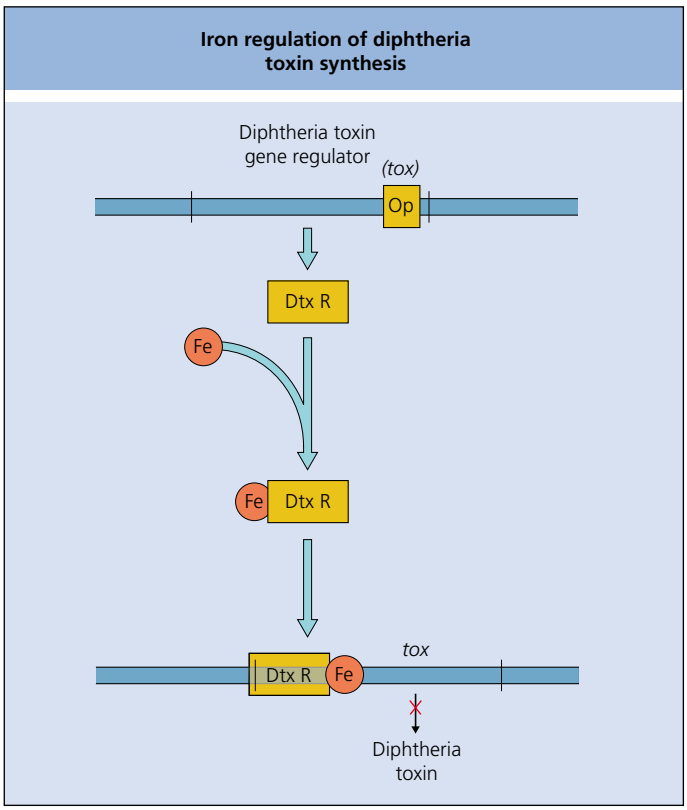


Fig. 1.19 Iron regulation of diphtheria toxin synthesis. High iron concentrations in the environment repress the synthesis of diphtheria toxin. When bound to iron, DtxR-Fe binds to the operator (Op) of the *tox* gene and acts as a transcriptional repressor of the *tox* gene. (Courtesy of Menno Kok & Jean-Claude Pechere.)

proteins that have a sialic acid in a 2,3 linkage to galactose residues at the termini of N- and O-glycan chains are not substrates for the host Ashwell receptor unless the terminal sialic acid is cleaved off. Pathogens such as *Strep. pneumoniae* that secrete a sialidase can remove sialic acids from host platelets that are then cleared from the circulation by the Ashwell receptor. This produces thrombocytopenia but avoids disseminated intravascular coagulation (DIC). Thus, one complication of severe pneumococcal sepsis is due to a secreted enzyme that is not generally considered to be an exotoxin.

Apoptosis

Apoptosis is a process in which the cell activates an intrinsic suicide program. It plays a key role in processes like organ development, tissue repair and maintenance of the dynamic equilibrium of the immune system. These processes critically depend on the generation of self-limiting organized structures through addition of new cells and elimination of 'old' cells. The morphologic changes associated with apoptotic death are a reduction of the volume of the cytosol and nuclear condensation (Fig. 1.20). The genome is fractionated by an endonuclease that cuts the DNA into multiples of 180–200 bp.[47] Finally, the remains of the cell are removed by phagocytosis without triggering an inflammatory response. In necrosis the cell does not participate actively in its own death and the dead cells trigger production of proinflammatory cytokines by macrophages.

Viral infection often triggers apoptosis of infected cells due to interruption of protein synthesis, transcription or signaling. For instance, apoptosis seems to contribute to the depletion of $CD4^+$ T cells, both in cell culture and in HIV-infected people.[48] Several different HIV proteins have been noted to both promote and inhibit apoptotic cell death.[49] Similarly, apoptotic cells have also been observed in infections caused by Epstein–Barr virus (EBV) and adenoviruses, though in latency EBV

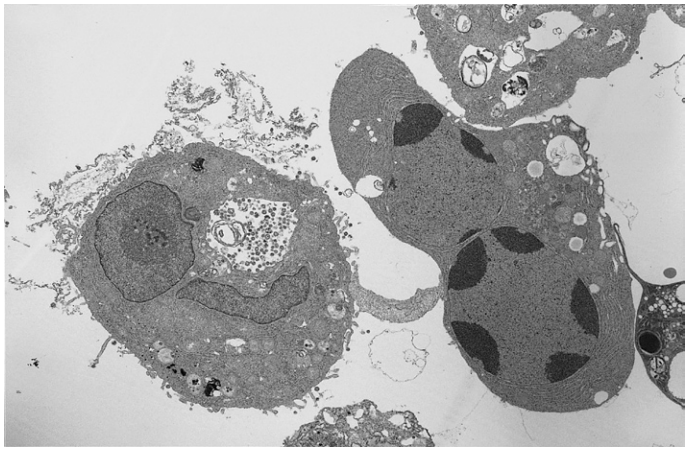


Fig. 1.20 Apoptosis induced by Sendai virus. Morphologic changes in the apoptotic Sendai infected cell (right) include the typical condensation of chromosomal DNA. (Courtesy of Menno Kok & Jean-Claude Pechere.)

latent membrane protein 1 (LMP-1) inhibits apoptosis.[50] Apoptosis can be seen to be beneficial to the host in that it can eliminate a host cell before it has produced a full complement of progeny virus.

Bacteria can also induce apoptosis. *Bordetella pertussis*, the agent of whooping cough, triggers macrophage apoptosis by interfering with cellular regulation at the level of the cytoplasmic second messenger cyclic AMP (cAMP).[51] The bacterium induces high levels of cytoplasmic cAMP, favoring the induction of apoptosis. *Shigella flexneri*, the etiologic agent of dysentery, can kill macrophages by apoptosis. Cell death is induced by invasion plasmid antigen B (IpaB) encoded by the *Shigella* virulence plasmid (see Fig. 1.14).[52] The *Shigella* IpaB protein binds to the host cytoplasmic enzyme interleukin (IL)-1b converting enzyme (caspase-1) and activates it.[53] Caspase-1 activates the proinflammatory cytokines IL-1 and IL-18 by proteolytic cleavage and initiates one of the proapoptotic pathways. In *Salmonella* infection the IpaB homologue SipB similarly activates caspase-1 to stimulate secretion of the proinflammatory cytokine IL-18 and induce apoptosis.[54] Timely induction of apoptosis in dendritic cells may well allow Salmonellae to exploit the mobility of these host cells to migrate away from the intestinal mucosa and establish systemic infection.

Virus-induced cytopathic effect

Many viruses damage the cells they infect, sometimes inducing visible and distinctive cytopathic effects (Fig. 1.21). Cytopathic effects may be mediated by either the presence of the virus or the host immune response. Poliovirus shuts off host cell protein translation through action of viral protease 2A resulting in cleavage of eIF4G needed for recognition of capped mRNA and cellular protein synthesis[55] and Coxsackie B virus protease 2A cleaves dystrophin in cardiac muscle, contributing to myocarditis.[56] In contrast, infection with hepatitis A and hepatitis C viruses produce very little direct killing of hepatocytes, with most liver damage mediated by the cytotoxic lymphocyte response.

Virus infection may also result in the intracellular accumulation or release of small molecules, such as reactive oxygen or nitric oxide, probably via effects on cellular signaling pathways or induction of innate immune responses. These may play important roles in cell destruction, particularly in macrophages. Rotavirus, CMV and HIV infection produce significant increases in intracellular calcium, a common pathway for the development of irreversible cell injury.

Viral fusion proteins mediate characteristic formation of multinucleated giant cells (syncytia). Examples include respiratory syncytial virus, parainfluenza viruses, measles virus, herpesviruses and some retroviruses. Viral infection can also produce eosinophilic or basophilic inclusion bodies in the cytoplasm or the nucleus. Inclusion bodies

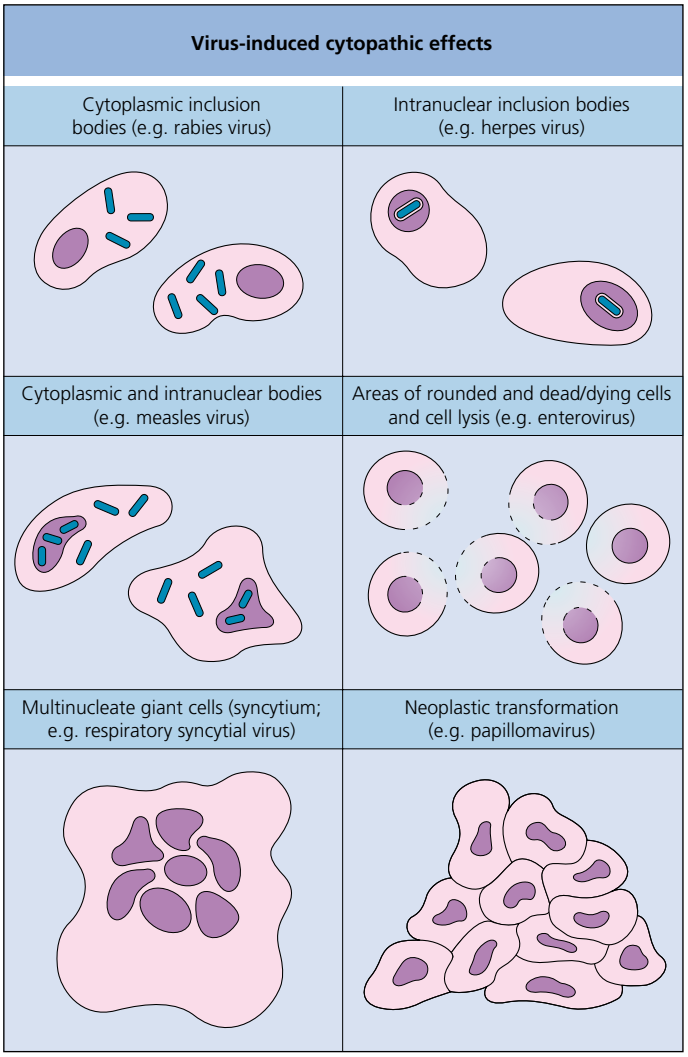


Fig. 1.21 Virus-induced cytopathic effects. (Courtesy of Menno Kok & Jean-Claude Pechere.)

represent aggregations of mature virions, sites of viral replication or assembly, or degenerative changes.

Infection and cancer

Infection can favor development of cancer by producing chronic inflammation, impairing immune surveillance and directly altering cell growth and death, for example:

- chronic *H. pylori* infection is associated with gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma;
- inflammation associated with *M. tuberculosis* infection may lead to adenocarcinoma of the lung; and
- *Schistosoma haematobium* infestation is associated with bladder cancer.

It is possible that different types of infection may act together in promoting neoplasia. For example, human papillomavirus (HPV) has been found to be present in all bladder tumors associated with schistosomiasis but only the minority without parasitic infestation. Expression of some viral genes (oncogenes) can drive cellular proliferation and impair apoptosis, producing disordered growth (transformation) that may lead to cancer. As examples, Burkitt's type lymphomas and craniopharyngioma are associated with EBV, cervical and anogenital carcinomas are associated with HPV, hepatitis B and C infection is associated with hepatocellular carcinoma, Kaposi's sarcoma-associated herpesvirus produces

Kaposi's sarcoma, and adult T-cell leukemia is caused by human T-cell lymphotropic virus type 1.[57]

In the case of HPV, persistent 'high-risk' infections (e.g. HPV16 and 18) may lead to the development of high levels of viral DNA, progressing to invasive carcinoma.[58] The HPV E2 protein regulates expression of E6 and E7 but is disrupted by viral integration, E5 acts as a viral analogue of the platelet-derived growth factor, E6 functions as a ubiquitin ligase to degrade the p53 anti-oncogene, and E7 binds to retinoblastoma protein (Rb) and cyclins A and E, allowing Rb phosphorylation and release of the E-2F, promoting G1 to S cell cycle transition.[59] With further chromosomal destabilization and mutations promoted by enhanced viral expression following DNA integration, the infected cells transform to a malignant phenotype.

Damage resulting from cytotoxic lymphocytes

The most effective host defense mechanism against most viral infections is mediated by the $CD8^+$ cytotoxic T lymphocytes (CTLs). The CTLs recognize, attack and lyse virus-infected cells that present viral antigens on their surface in the context of MHC class I molecules. In addition to CTLs, natural killer lymphocytes can kill virus-infected cells. The cytotoxic reaction contributes to the pathologic and clinical picture of many viral diseases. The characteristic measles rash is produced by infiltration of lymphocytes including CTLs attacking skin cells infected by the measles virus. This explains why children with defects in cell-mediated immunity do not develop a rash during measles infection. In this disease, rash indeed represents a good immune response by the host, whereas its absence may signal uncontrolled viral growth. As previously noted, hepatitis due to hepatitis A and C may be largely or entirely the consequence of immune attack rather than viral cytopathic effects. It is also believed that lymphocyte-induced cytotoxicity contributes to the pathology associated with persistent virus infections such as the subacute sclerosing panencephalitis caused by defective measles virus within the brain.

Harmful immune responses

The destructive potential of the immune system is considerable. It can damage the host in a variety of ways.

Autoimmunity

Autoimmune reactions break the rules of the 'self versus nonself' dichotomy. Autoimmune reactions, directed against 'self-proteins', may result from partial identity of antigenic determinants of the host and an infective agent or from alterations of self-components caused by infection. Acute rheumatic fever occurring after group A streptococcal pharyngitis has been associated with antibodies against antigens found in the cell wall of the streptococcus that also recognize components of the endocardium and the joint synovial membrane molecules and thus induce an autoimmune response. Mycobacterial infection may give rise to antibodies and T cells that are reactive to both the microbial (nonself) and the host (self) heat shock proteins.

Another example of molecular mimicry is the association between production of antiganglioside antibodies, the Miller-Fisher (MFS) variant of the Guillain–Barré syndrome (GBS), and prior *Campylobacter jejunii* infection. This illness is almost certainly due to cross-reacting antibodies against the sialated LPS of *C. jejunii*.

Hypersensitivity reactions

Hypersensitivity reactions occur if the host immune system seemingly overreacts to microbial infection. Hypersensitivity reactions have been classified by Gell and Coombs into four types.

Type I or immediate hypersensitivity

Type I hypersensitivity occurs within minutes of antigen exposure. It results from antigen binding to mast cell-associated IgE. Vasoactive amines are released and anaphylactic reactions may develop. Certain forms of rash after helminth infections seem to be due to this type of hypersensitivity.

Type II or cytotoxic hypersensitivity

Type II hypersensitivity is a consequence of the binding of specific antibodies to cell surface-associated antigens. Antibody binding mediates cytotoxicity via complement activation or natural killer cells. Thus cells bearing microbial antigens may be lysed via an antibody-dependent mechanism. Such a mechanism has been suggested to account for liver cell necrosis during hepatitis B infection.

Type III or immune complex-mediated hypersensitivity

Type III hypersensitivity is induced by classic complement activation, caused by extracellular antibody–antigen complexes. This causes inflammation and changes in vascular permeability and attracts neutrophils to tissues where the immune complexes are deposited, including the kidneys, joints and small vessels of the skin. Glomerulonephritis in malaria and subacute endocarditis are probably due to this mechanism.

Type IV or delayed-type hypersensitivity

Type IV hypersensitivity typically occurs at least 48 hours after exposure to an antigen. It involves activated T cells, which release cytokines, macrophages attracted by these cytokines, and cytotoxic $CD8^+$ T cells. Delayed-type hypersensitivity and granuloma play a major role in tissue damage observed during infections with slow-growing intracellular organisms, such as *M. tuberculosis* (tuberculosis), *M. leprae* (leprosy) and *Histoplasma capsulatum*. Many of the clinical manifestations of chlamydial disease, in particular trachoma, seem to result from a delayed-type hypersensitivity triggered by chlamydial heat shock proteins. In spite of the involvement of bacterial heat shock proteins, this is not an autoimmune phenomenon, because the unique rather than the conserved portions of these proteins seem to be implicated here.

Superantigens and bacterial components associated with toxic and septic shock

Toxic shock and septic shock are exceptionally impressive syndromes associated with a variety of infectious diseases. Severe hypotension, multiple organ failure and intravascular disseminated coagulopathy occur in the most severe cases. Pathogenesis of these syndromes is complex. Various bacterial components, including LPS, peptidoglycans, lipoteichoic acid and (in some cases) exotoxins acting as superantigens (see Table 1.7) trigger an intense, potentially lethal host response. Macrophages, neutrophils and/or T cells play important roles in the cascade of events leading to this condition (see Chapters 9 and 44) by releasing high levels of inflammatory response mediators, notably tumor necrosis factor and IL-1.

How micro-organisms escape host defense

In spite of the efficacy of host defense mechanisms, microbial pathogens can still infect humans and cause disease. This is in part due to the very potent weapons micro-organisms have (a single gram of crystalline botulinum toxin could potentially kill more than 1 million people) but it is also due to the intricate strategies that micro-organisms use to evade host defenses (Table 1.8).

Surviving the phagocyte and complement attack

Immediately after passage through the epithelial surface, the invading micro-organism encounters the most powerful actors of host defense: phagocytes. The two main types of phagocyte are PMNs and macrophages. These 'professional' phagocytes can bind micro-organisms

Table 1.8 Evasion of host defenses

Mechanism	Examples
Surviving the phagocyte and complement attack	
Inhibition of chemotaxis	C5a peptidase by *Streptococcus pyogenes*
Killing the phagocyte before ingestion	α-Toxin and Panton–Valentine leukocidin by *Staphylococcus aureus*
Avoiding ingestion	Bacterial capsules (e.g. *Streptococcus pneumoniae*) K (capsule) and O (LPS) antigens in Gram-negative rods
Avoid complement lysis	Coating with IgA antibodies (*Neisseria meningitidis*) Porin binding factor H and C4 binding protein (*N. gonorrhoeae*) M protein (*Streptococcus pyogenes*)
Surviving within phagocytes	Inhibition of phagolysosome fusion (*Chlamydia trachomatis*) Escape from phagolysosome (*Listeria monocytogenes*) Inhibit NADPH oxidase fusion with phagosome (*Salmonella typhimurium*) Inhibition of acidification of phagosome due to exclusion of the vacuolar H^+-ATPase (*Mycobacterium tuberculosis*)
Antigenic variations	Shift and drift in influenza A virus, pilin variation in *N. gonorrhoeae*
Tolerance	Prenatal infections
Immunosuppression	
Destroying lymphocytes	Depletion of $CD4^+$ cells by HIV
Proteolysis of antibodies	IgA protease by *Haemophilus influenzae*
Presence in inaccessible sites	Latent infection in dorsal root ganglia (herpes simplex virus)

with a variety of receptors, some of which specifically interact with bacterial surface structure or with antibodies or complement bound to the microbial surface (opsonized micro-organisms). The micro-organisms usually pass into the epithelial cell via phagocytosis or pinocytosis, although some (especially viruses) may enter the cytosol directly.

Bacteria invariably go through an endosomal stage, in which they will be exposed to a multitude of phagocyte defense mechanisms such as acidification, reactive oxygen species, bactericidal peptides, and hydrolytic enzymes released after phagosome–lysosome fusion. In addition, in the endosomal pathway, micro-organisms are deprived of the nutritional wealth of the cytosol. If the pathogens are killed and degraded their microbial antigens may be presented to lymphocytes. However, micro-organisms have developed strategies to avoid, mislead, deregulate or even profit from phagocytes.[60] Organisms like *Salmonella* require acidification of the phagosome and low Ca^{2+}/Mg^{2+} concentrations to trigger the PhoP/PhoQ transcriptional regulatory system that is required for survival inside macrophages. If acidification is prevented, *Salmonella* are killed by macrophages. In contrast, *M. tuberculosis* prevents acidification of the phagosome to varying degrees, and those micro-organisms that reside inside less acidic phagosomes remain metabolically active.

Inhibition of the mobilization of phagocytes

Extracellular micro-organisms can avoid phagocytes by inhibiting chemotaxis or complement activation (see below). A bacterial enzyme that degrades complement protein C5a, a main chemoattractant for phagocytes, has been discovered in *Strep. pyogenes* and *agalactiae*. Pertussis toxin catalyzes ADP ribosylation in neutrophils, which causes a rise in intracellular cAMP levels that ultimately impairs chemotaxis. *Yersinia pestis* employs several secreted enzymes (YOPS) to subvert macrophage phagocytosis and thus remain extracellular.

Killing the phagocytes before being ingested

Many soluble products excreted by bacteria are potentially toxic for phagocytes entering the foci of infection. Streptolysin-O binds to cholesterol in cell membranes, which results in rapid lysis of PMNs. In the process, the lysosomes are also disrupted and release their toxic contents, which may have deleterious effects on the neighboring cells. Other examples of toxins that are directed against phagocytes include the γ toxin of *Clostridium perfringens*, α toxin and Panton–Valentine leukotoxin made by *Staph. aureus*; the latter specifically targets myeloid cells and has species specificity. Many so-called extraintestinal pathogenic *E. coli* are hemolytic because they produce an RTX membrane toxin that damages PMNs, impairing their function in several ways, depending on the local concentration of the toxin. Several toxins from *Clostridium perfringens* produce similar effects. Indeed, pus sampled from gas gangrene may contain numerous Gram-positive rods without any visible PMNs.

'Professional' phagocytes as vectors or refuges

Legionella pneumophila provokes entry in mononuclear phagocytes by accumulating complement factor C3bi on the envelope of the organism. This complement factor is a ligand for the phagocyte receptor CR3, and enhances phagocytosis. Following uptake, *Legionella* remains in the phagosomes, which do not fuse with lysosomes and thus provide protection. Alveolar macrophages are host cells for *M. tuberculosis*.[61] *Salmonella enterica* are ingested by intraepithelial dendritic cells and carried into regional lymph nodes and the systemic circulation by those cells. Many viruses (HIV, dengue virus, measles, etc.) infect and replicate in monocyte macrophages. Infected monocytes may provide HIV with a route through the blood–brain barrier into the CNS[62] and dendritic cells loaded with infectious HIV may activate and infect T cells[63] (see Chapters 120 & 121). *Ehrlichia*, which are small, Gram-negative, obligatory intracellular bacteria, directly infect the cytoplasm of granulocytes or macrophages, depending on the bacterial species. All organisms that have adapted to live inside phagocytic cells have developed mechanisms to escape, disarm or survive the onslaught of antimicrobial factors.

Avoiding ingestion

The surface of numerous pathogenic bacteria is covered with a loose network of polymers, which constitutes the bacterial capsule.[64] Capsular material may be very thin, visible only by electron microscopy, as is the case with the hyaluronate capsule of *Strep. pyogenes*. In some species (*Strep. pneumoniae, Klebsiella pneumoniae*) capsule material is abundant, easily visible with a light microscope and responsible for a mucoid aspect of the bacterial colonies. Most of the capsules are composed of polysaccharides, others are made of proteins or a combination of carbohydrate and protein. Some capsule contents mimic host polysaccharides and are thus recognized as 'self' by the host immune system. Examples are the capsules of *N. meningitidis*, which contain sialic acid, and *Strep. pyogenes*, which contain hyaluronic acid. Proteins that envelop bacteria in S-layers can serve the same function as polysaccharides, as in the cases of *Campylobacter fetus* and *Bacillus anthracis*.

Capsules may protect bacteria from complement activation.[65] As a result, encapsulated bacteria are not immediately recognized as invaders by the phagocytes. Capsulated *Strep. pneumoniae* resist engulfment by macrophages and PMNs and are virulent; however, noncapsulated strains are easily phagocytosed and are avirulent.[66]

Meningococci circulating in the blood are coated with IgA, which is not an activator of the complement cascade. *Schistosoma mansoni* incorporates decay accelerating factors in its membrane; these are host plasma proteins that inhibit deposition of C3 onto host cell membranes. Activation of complement in the blood is thus avoided by the parasite.

Matrix (M) proteins, which form fibrillae (see Fig. 1.11), are considered to be the primary virulence determinants of *Strep. pyogenes*. Matrix protein renders the bacteria resistant to phagocytosis by human neutrophils. Matrix fibrillae are approximately 50–60 nm in length and exhibit a seven-residue periodicity. They exist as stable dimers, arranged in an α-helical, coiled coil configuration, with the carboxyl terminal portion closely associated with the cell wall (see Fig. 1.11). Streptococci that express M proteins on their surface are poorly opsonized by the alternative pathway and resist PMN phagocytosis. In contrast, streptococci that fail to express M protein are readily opsonized and phagocytosed, and are avirulent. Resistance to phagocytosis can be overcome by antibodies directed against type-specific M epitopes.

The mechanism of antiphagocytic activity of M proteins is still unclear. According to one hypothesis, fibrinogen, known to bind to M protein, may hinder access to complement-binding sites on the bacterial surface, disguising the pathogen as 'self'. In another hypothesis, a complement control protein (protein H), which also binds M, may be responsible for the observed complement resistance of virulent *Strep. pyogenes*.

Survival within phagocytes

Once ingested by the phagocyte, the pathogen may survive and grow using a variety of strategies (Fig. 1.22). Some microbes prevent exposure to hydrolytic enzymes by inhibiting fusion of the phagosome and the lysosome, others survive within the phagolysosome because they resist enzymatic degradation or neutralize toxic products to which they are exposed in this compartment. Some bacterial pathogens (such as Salmonellae discussed above) extensively modify endosomes into customized survival vesicles. Certain types of bacteria rapidly escape from the phagolysosome and propagate in the cytoplasm, as described above for *Listeria monocytogenes*. Recent studies suggest that intracellular pathogens, notably *M. tuberculosis*, may inhibit the early host response at the level of host gene expression.

Inhibition of phagolysosomal fusion

Salmonella spp. have developed several strategies to survive and propagate in macrophages; *Salmonella* spp. that lack this capacity to survive in macrophages are avirulent. Several hours after infection *in vitro*, two distinct *Salmonella* populations can be seen in the macrophage. One consists of rapidly dividing bacteria located in large unfused phagosomes. This population may grow rapidly and kill the macrophage, leading to the liberation of intracellular bacteria.[67] *In vivo*, this population may be responsible for the acute stage of salmonellosis.

The second population of *Salmonella* consists of nondividing organisms located in phagolysosomes. This population resists the toxic effect of lysosomal products and is believed to account for the prolonged survival of *Salmonella* spp. in the body. Long-living stromal macrophages of the bone marrow may act as long-term reservoirs and be responsible for the relapses of salmonellosis that are seen in immunosuppressed patients with AIDS. The dormant phase represents a well-regulated physiologic condition associated with nutrient deprivation *in vitro*.

Inactivation of reactive oxygen species

Reactive oxygen species damage DNA and inhibit bacterial oxidative phosphorylation. Bacteria may escape from the damaging effect of reactive oxygen species by rapid detoxification of the bactericidal products and by efficient DNA repair. Several bacterial pathogens produce superoxide dismutase (SOD) and catalase, two enzymes that might eliminate the reactive oxygen species and damage to DNA may be efficiently repaired through a RecA-dependent pathway. In Salmonellae the RecA pathway seems to be more important, as *rec*A mutants are avirulent. However, enzymes that inactivate oxygen radicals are also virulence factors.

A SOD encoded by a bacteriophage that is present in many virulent *Salmonella enterica* is expressed under the control of the sigma factor rpoS when the bacteria are inside phagocytes. In contrast, expression of the chromosomally encoded SOD that is in all strains of *Salmonella enterica* is controlled by another sigma factor and expression is repressed in intracellular bacteria. However, the ability of this bacterial species to modify the endocytic pathway of the host cell seems to be the most important mechanism of resistance to reactive oxygen species. In macrophages, virulent Salmonellae localize in phagosomes devoid of NADPH oxidase, the enzyme that drives the respiratory burst.[68]

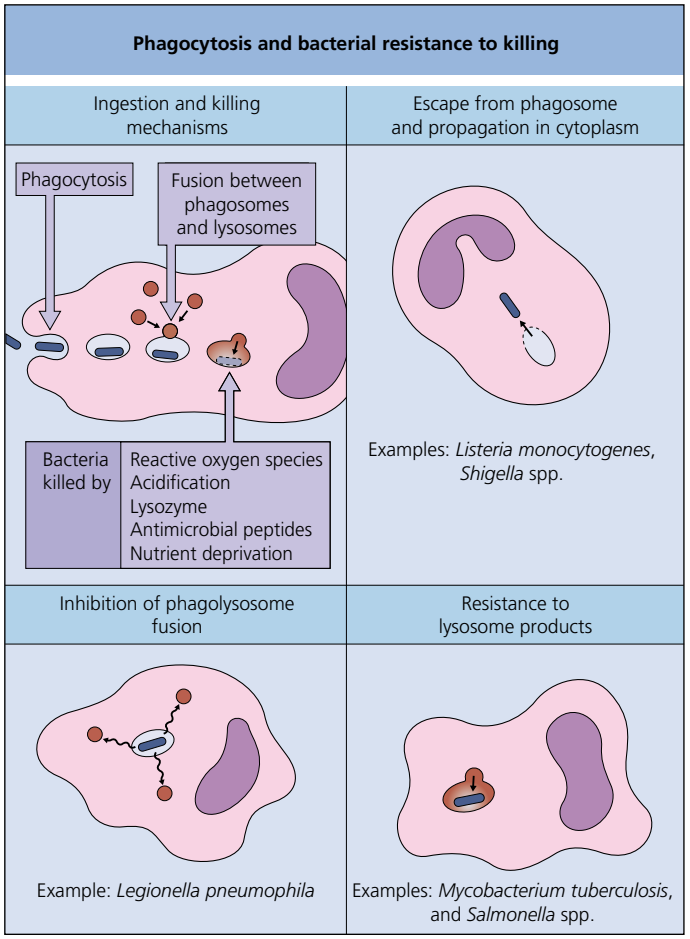


Fig. 1.22 Phagocytosis and bacterial resistance to killing. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Resistance to antimicrobial peptides

Several cationic peptides are produced within the lysosomal granules and are believed to kill intracellular pathogens by forming channels in the bacterial cell wall. *Salmonella* spp. resist these antimicrobial peptides by at least two complementary mechanisms, one of which, encoded by the *sap* locus, is characterized in some detail (Fig. 1.23). It seems that the SapA protein forms a complex with the antimicrobial peptides, reducing the deleterious effect on the bacterial membranes.

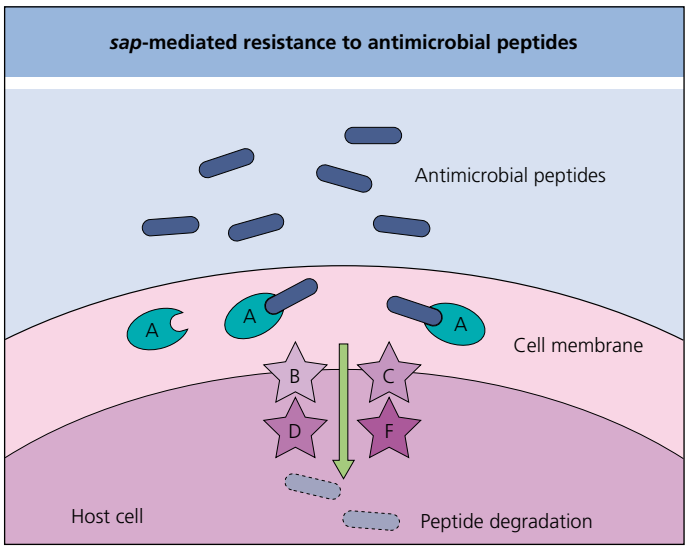


Fig. 1.23 Mechanism of resistance to macrophage antimicrobial peptides by *Salmonella* spp. *Salmonella* produces the SapA (A) peptide, which complexes with host cell antimicrobial peptides. Other proteins encoded by the *sap* locus (SapB, SapC and SapD) are required for the transport of the SapA-antimicrobial peptide complex into the cytosol where the antimicrobial peptide is degraded. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Other proteins encoded by the *sap* locus (SapB, SapC and SapD) allow the transport of the SapA–peptide complex into the cytosol. Within the cytosol, peptidases degrade the antimicrobial peptides. Recently it was shown that pili make group B streptococci resistant to Mouse CRAMP and the human homologue LL-37.

Antigenic and phase variations

A powerful survival strategy for a pathogen would be to mislead the specific host immune response by 'changing appearances'. Three examples of molecular mechanisms used to achieve antigenic variation, one each by a bacterium, a virus and a protozoan, are illustrated below (Table 1.9).

Table 1.9 Examples of antigenic variations

Genetic mechanisms	Examples
Recombination between different copies of pilin genes	Pili in *Neisseria gonorrhoeae*
Phase variation – turning expression of an antigen on or off ('flip-flop')	Flagella in *Salmonella*; pili in *N. gonorrhoeae*
Gene reassortment between two strains infecting the same cell	Influenza virus type A
Mutation of surface antigens	Influenza virus type A, B and C, deletion of flagella in *Shigella* spp. (avoids TLR5 signaling)
Gene switch leading to surface glycoprotein changes	*Trypanosoma brucei*
Gene switch leading to production of nonactivating lipopolysaccharides	*Yersinia pestis* growing at 37°C; *Salmonella enterica* growing inside phagosome (PhoP regulated)

Antigenic variation in *Neisseria gonorrhoeae*

Neisseria gonorrhoeae varies the composition of at least three major components of its outer membrane: the pili, which mediate the initial attachment to host cells; the membrane protein P.II, responsible for closer attachment resulting in phagocytosis; and LPS, described earlier.

Antigenic variations in the major pilin subunit are essentially due to recombination between different copies of *pil* genes scattered over the chromosome (Fig. 1.24). Only one or two of these are expressed (*pil*E, where E denotes 'expressed') at any point in time, but an array of antigenically distinct pili may be produced in response to an antibody challenge. In addition to this mechanism, pili are subject to phase variation (i.e. switches between *pil*-positive and *pil*-negative variants). Phase variation is controlled at the transcriptional level.

The P.II protein is similarly subject to genetic variation. As a consequence, the specific immune response never quite catches up with genetic variation in the bacterial population. The combination of this mechanism, LPS sialylation (see above) and IgA protease production, explains the apparent lack of acquired immunity to gonorrhea and makes vaccine development very difficult.

Shift and drift in influenza A viruses

Every year, during seasonal influenza, vaccination programs are confronted with the problem of antigenic variation. Influenza viruses change through drift and shift. Antigenic drift refers to the gradual accumulation of point mutations during annual circulation of influenza as a consequence of the high error rates associated with RNA-dependent RNA polymerase during virus replication. Influenza A virus mutants with antigenic changes tend to have a selective advantage over the nonmutant viral population. The rapidity with which drift can produce change is illustrated by the dramatic increase in amantadine resistance (from 2–12% to >91%) in influenza A/H3N2 strains, associated with a single mutation at position 31 of the M2 protein from 2005 to 2006. As a consequence of antigenic drift, the composition of the influenza vaccine must be evaluated very carefully and updated on an annual basis in order to offer coverage for the strains likely to be circulating at the time.

Antigenic shift refers to the emergence of a novel influenza virus in humans, due to direct introduction of an avian strain or to a new strain produced by recombination and reassortment of two different influenza viruses. Antigenic shift results in dramatic changes in the antigenic composition of the surface hemagglutinin (which binds the host cell receptor) or the neuraminidase (which modifies these receptors) and can cause devastating worldwide epidemics, or pandemics, in the immunologically unprepared population. Recent influenza A pandemics occurred in 1957 (the H2N2'Asian Flu') and 1968 (the H3N2'Hong Kong Flu'). An outbreak of avian influenza from exposure to infected poultry in Hong Kong in 1997 caused 18 human deaths. A genetically different strain of A/H5N1 circulated in domestic birds throughout Asia, causing 387 cases and 245 deaths between 2003 and 2008, raising concerns that a new pandemic might arise. Instead, in 2009, an unanticipated, novel H1N1 reassortant influenza A virus with origins in circulating seasonal influenza, avian, and both classic and Eurasian swine strains emerged to cause the latest worldwide influenza A pandemic.[70]

Antigenic variations in *Trypanosoma brucei*

African trypanosomes (*Trypanosoma brucei*) are flagellated protozoa, transmitted to humans by several species of *Glossina* (tsetse). The parasite survives in mammalian body fluids thanks to antigenic variation of the variant surface glycoprotein (VSG), which forms a 15 nm thick monolayer covering most of the parasite surface.[69] Within a single generation, most or all of the 10^7 VSG molecules may be replaced by an unrelated species, stemming from a repertoire of an estimated 1000 genomic copies of the gene. The VSG gene is invariably expressed from a polycistronic transcription unit, in the

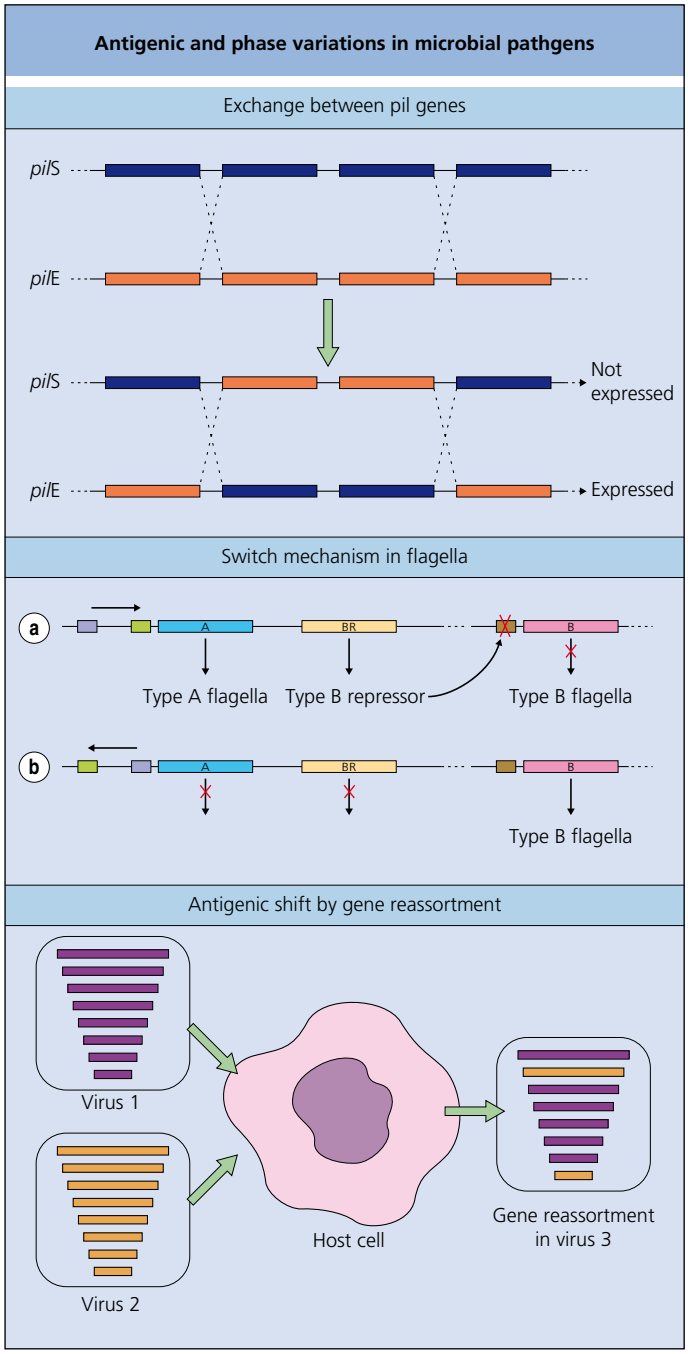


Fig. 1.24 Antigenic and phase variations in microbial pathogens. Three mechanisms are shown. (Top) Exchange of DNA between nonexpressed copies of *pil*S and the expressed gene *pil*E in *Neisseria gonorrhoeae* can change the expressed antigen. (Middle) A switch mechanism is responsible for the (mutually exclusive) production of type A and type B flagella in *Salmonella typhimurium*. Phase variation depends on the orientation of a DNA fragment adjacent to the type A flagella gene. When A is expressed (a) from the promoter in the invertable fragment, the repressor for the type B flagella is expressed at the same time. As a consequence the type B flagella gene is repressed. Inversion of the DNA fragment abolishes expression of the A-repressor gene and the B-repressor gene (b). In this situation type B flagella are produced. (Bottom) Antigenic shift by gene reassortment results from infection of a single cell by two different virions. (Courtesy of Menno Kok & Jean-Claude Pechere.)

so-called telomeric expression site adjacent to the telomeric repeats. During chronic infection, patients experience successive episodes of parasitemia, each episode coinciding with the expression of a new VSG on the surface of the parasite. With this strategy, trypanosomes avoid complete eradication by the specific immune response, while maintaining the pathogenic burden at sublethal levels. The closely related *T. brucei brucei*, which causes the bovine disease nagana, does not spread to humans because it is sensitive to high-density lipoprotein in human serum.

IMMUNOSUPPRESSION

The most illustrative example of immunosuppression induced by microbial infection is provided by HIV. Human immunodeficiency virus circulating in the bloodstream readily infects $CD4^+$ lymphocytes, macrophages and dendritic cells. The destruction of $CD4^+$ T-helper cells is particularly detrimental to the host and accounts for the emergence of a variety of opportunistic infections as soon as the T-cell count drops below a critical level. In addition to its general immunosuppressive effects, HIV-1 preferentially infects HIV-1 specific $CD4^+$ T cells, thereby undermining the ability of the host to mount an effective immune response to the virus itself.[71] It has recently been shown that HIV infection rapidly causes a profound depletion of $CD4^+$ T cells in the gut as well as the lymph nodes and peripheral blood. This local immunosuppression may in turn be instrumental in producing continued T-cell activation due to increased bacterial translocation through the damaged mucosal barrier, increasing susceptibility to additional HIV infection.[72]

Other viruses may produce immunosuppression in a more subtle fashion. Measles virus infects macrophages and both B and T lymphocytes, interfering with the immunocompetence of the host for weeks after resolution of clinical disease. As a consequence, in areas with a high prevalence of tuberculosis, measles epidemics may be followed by outbreaks of tuberculosis. Gonococci, meningococci and *Haemophilus influenzae* produce proteases that hydrolyze secretory IgA1 antibodies. Protease-negative mutants of these bacterial strains are less virulent, suggesting a role for mucosal IgA1 antibodies in host defense against these pathogens.

CONCLUSION

Throughout evolution, humans, like all mammalian species, have maintained an intimate relationship with the microbial world. We have survived thanks to the efficient defense mechanisms we have developed against potentially dangerous micro-organisms. Pathogenic micro-organisms are still here because they have found ways of avoiding elimination by their host or by the microbial competition. 'Successful' pathogens have developed strategies to enter the body and reach and colonize their favorite niche, while defying the powerful human immune system.

In this chapter we have looked into microbial survival strategies. Although some of these have been analyzed in 'molecular detail', a lot remains to be discovered. Future remedies for infectious diseases are likely to be aimed at specific molecular interactions between the pathogenic micro-organism and its host.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com