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Host defence

Immunity is concerned with recognizing and disposing of foreign material entering the body. Our defence against infection starts with physical barriers such as skin, mucous membranes etc. If these are breached, the consequent immune response falls into two distinct yet interacting branches: the innate and the adaptive immune systems. Both have cellular and humoral (antibody-mediated) components.

Innate defence mechanisms

It takes time to raise an adaptive response. The innate system provides a high-speed, on-the-scene reaction to foreign material. Its main components are complement proteins, phagocytes, and natural killer cells. The cytokines (proteins released by cells that influence other cells) and chemokines (chemicals that attract cells) produced by these players initiate inflammation, and both activate and control the manner of the adaptive response.

Complement pathways

One of the oldest parts of the immune system (sea urchins have had it for 700 million years), it comprises around 20 different protein that operate in cascade to destroy invaders and signal other immune system components. It can be activated in three ways:

- classical pathway – activation by antibody bound to its antigen. Immunoglobulin M (IgM) is particularly good at this, which is logical since it is one of the early antibodies made by the adaptive system
- alternative pathway – unlike the classical this is non-specific. One of the complement proteins, C3b binds amino or hydroxyl groups on, for example, bacterial cells. Once stabilized in this way it is able to activate the cascade
- lectin activation pathway – mannose-binding lectin (a lectin is a protein that binds carbohydrates), present in the tissues and circulation binds mannose on the surface of, for example, a bacterium. This triggers the production of C3b with the consequences described above.

The activated complement cascade produces membrane attack complexes (MAC), which punch holes in the surface of the foreign organism. In addition, complement proteins opsonize invaders facilitating phagocytosis, and act as chemoattractants for other immune system players.

Phagocytes

Macrophages and neutrophils are the key innate phagocytes. Macrophages can live for months and are based in tissues. Their role is that of sentinel – continually phagocytosing, watching for invaders, and signalling attacks. When activated they can present antigen to T cells. Neutrophils comprise around 70% of circulating white blood cells. In the blood they are inactive. In response to cytokines indicating a local immune response, they leave the circulation and become active, highly phagocytic killing machines.

Natural killer cells

Natural killer (NK) cells are in the same family as lymphocytes. They leave the circulation at sites of infection and can kill tumour cells, virus-infected cells, parasites, and fungi. They act by injecting enzymes into the target cell, or by interacting with a protein called 'Fas' on the surface of a target, prompting 'suicide'. They may be activated by substances such as lipopolysaccharide (LPS) from bacterial cell walls, and by interferon- α and - β from virus-infected cells. They are able distinguish self from foreign to a limited degree.

Adaptive defence mechanisms

B cells

B lymphocytes are produced and mature in the bone marrow. Each is able to produce only one antibody. It does this by rearranging the deoxyribonucleic acid (DNA) encoding the antibody into one of an estimated 100 million possibilities. This antibody is displayed on the cell surface as the B cell receptor (BCR). Activation of a naive B cell requires binding of the BCR to its cognate antigen and (generally) a co-stimulatory signal (usually provided by a helper T cell). It then proliferates to produce a clone of identical B cells, most of which mature into antibody factories: plasma B cells. Others may become memory cells.

Antibodies

These large proteins (immunoglobulins) opsonize foreign material, marking it for phagocytosis. Some are able to bind and block the proteins on a virus' surface required for uptake into cells, 'neutralizing' antibodies. Some are specific for toxins produced by an infecting organism. There are four main classes, each with an antigen-binding region (Fab) and a constant 'tail' region (Fc) (Fig. 1.1). Variations in the Fc region determine the class, its function, and to which immune system cells it will bind. The class a B cell produces is determined by the cytokine environment, particularly those from T helper cells.

- IgM – the first antibody (Ab) made when naive B cells activated. Like a pentameric IgG it is very effective at activating complement, and at viral neutralizing.
- IgG – 75% of Ab in the blood. Good at opsonizing, neutralizing viruses and can increase NK cells' cytotoxic activity (antibody-dependent cell cytotoxicity). Longest-lived Ab (half life about 3 weeks) and able to cross the placenta.
- IgA – the most abundant Ab in the body (as opposed to circulation). Protects all mucosal surfaces. Much like a dimeric IgG, it has four binding sites and can bind pathogens into clumps aiding their expulsion (e.g. in faeces). Resistant to acid and enzymes in gastrointestinal tract. Secreted in breast milk. No complement activity.
- IgE – role in defence against parasites. Pathological role in anaphylactic shock and allergy.

T cells

T lymphocytes are produced in the bone marrow but mature in the thymus. They bear T-cell receptors of similar variety to BCR and produced by a comparable DNA-juggling mechanism. Unlike BCR (which can recognize nearly any organic molecule), they recognize solely protein antigens and only if presented by another cell in association with a major histocompatibility complex (MHC) molecule. There are two main types of T cell:

- cytotoxic T lymphocyte (CTL) – recognizes Ag presented on MHC class I molecules which are found on nearly all cells in the body. MHC class I acts much like an advertising hoarding, displaying to the CTL what is 'on' inside the cell, be it benign native proteins, or foreign material (e.g. a cell producing viral protein). CTLs can then make contact with

potentially infected cells and trigger their demise. CTLs bear the MHC class I co-receptor, CD8

- helper T cell (Th cell) – recognize Ag presented on MHC class II which is made only by certain 'antigen-presenting cells' (APCs). The proteins presented are derived from material the APC has taken up from its environment – e.g. bacterial proteins, opsonized and phagocytosed viral particles. Th cells bear the MHC class II co-receptor, CD4.

Th cells are activated when their TCR recognizes its Ag–MHC and the cell receives a co-stimulatory signal – this is the role of APCs: macrophages, dendritic cells, and activated B cells. Once properly activated, they begin dividing to produce a clone of cells. These produce cytokine mixes appropriate to the insult (e.g. in a viral infection the cocktail might stimulate increased B-cell production of IgG, and activate CTLs and NK cells).

Virgin CTL activation requires T-cell receptor (TCR)/Ag–MHC recognition and help from a helper T cell. Once activated, the CTL proliferates and the offspring move to the area of infection where they identify and kill infected cells.

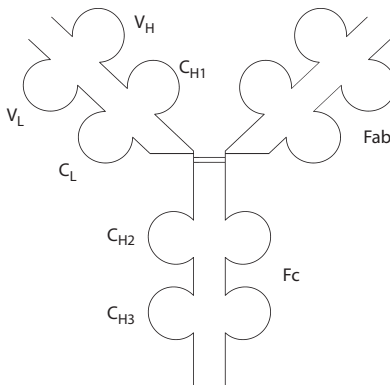


Fig. 1.1 Antibody structure. Immunoglobulin consists of a light chain containing a variable domain V_L and a constant domain C_L . The heavy chain consists of one variable V_H and three constant domains C_{H1-3} . The segment containing the C_{H2-3} of the heavy chains is the F_C portion and the two segments containing V_L , V_H , C_L , and C_H are each termed Fab fragments. Reproduced from Wilkins et al. *Oxford Handbook of Medical Sciences* (2005), with permission from Oxford University Press.

Immunity against viruses

The key process in viral infection is intracellular replication, hence the adaptive system and CTLs play a key role. The immune system can subvert the virus in a number of ways as detailed in the following text and in Fig. 1.2.

Defence against viral cell entry

- Antibodies may bind viral surface antigens required for cell entry, thereby neutralizing the virus (1).

Reducing the chance of cell infection

- Virus infection stimulates the production of interferons, a group of proteins which stimulate cells to block transcription of virus, and thus protect them from infection (7).

Recognizing and destroying virus-infected cells

- In a new infection, CTL recognize foreign viral peptides presented by MHC class I on the surface of infected cells (2). They are activated if they experience a co-stimulatory signal from a Th cell and proliferate. These activated CTLs can then trigger cell death on future encounters with their specific Ag-MHC complex (6)
- Certain viruses cause a reduction in surface expression of MHC I in cells they have infected as a means of avoiding CTLs. NK cells become suspicious of cells with absent MHC I and are capable of killing these cells early in infection (8).

Extracellular virus

- Virus enters the circulation from infected cells. Viral components presented on APCs activate Th cells (3), enabling them to produce appropriate cytokines to coordinate the immune response, recruit further cells, and provide co-stimulatory signals for CTLs' and B cells' activation.
- B cells are activated on recognizing their cognate Ag (4) together with T-cell help. They proliferate into plasma cells (5) producing antibody. Some form memory.

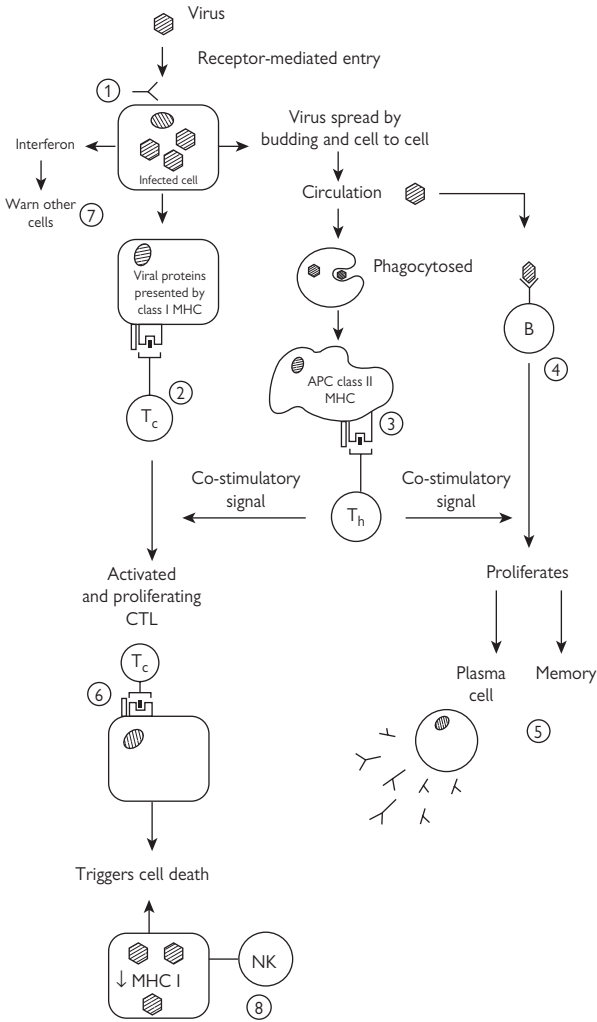


Fig. 1.2 Overview of viral defence.

Immunity against bacteria

Most bacterial infections, e.g. a boil, are localized and dealt with by cells residing in the affected tissue (e.g. macrophages) and those recruited by the consequent inflammatory response. In bacteraemia, the response is correspondingly greater and the inflammatory response more severe. The complement proteins and humoral immune response are the key players (Fig. 1.3).

Innate immune strategies

- The high-speed innate response may be all that is required to destroy a few infecting bacteria (e.g. from a splinter).
- Lysozyme is a 'natural antibiotic'. It acts on peptidoglycan in the bacterial cell wall causing lysis (2).
- Complement proteins may lyse bacteria directly with membrane attack complexes (see above) or facilitate phagocytosis by opsonizing the cell (3, 4).
- Macrophages and other phagocytic cells eat bacterial cells either directly or aided by opsonization with complement or specific Ab (4). Once inside the cell they are destroyed by toxic enzymes.

Adaptive immune strategies

- Macrophages can present bacterial protein components on MHC class II activating specific Th cells (6). These produce cytokines recruiting and activating other cells.
- Specific B cells recognize Ag and with T-cell help activate and proliferate (7). The Ab produced may be to structural components on the surface (helping complement and phagocytosis), or may neutralize toxins produced by the bacteria (e.g. tetanus). Endotoxins are constituents of the cell wall (e.g. LPS); exotoxins are specifically secreted products (1).

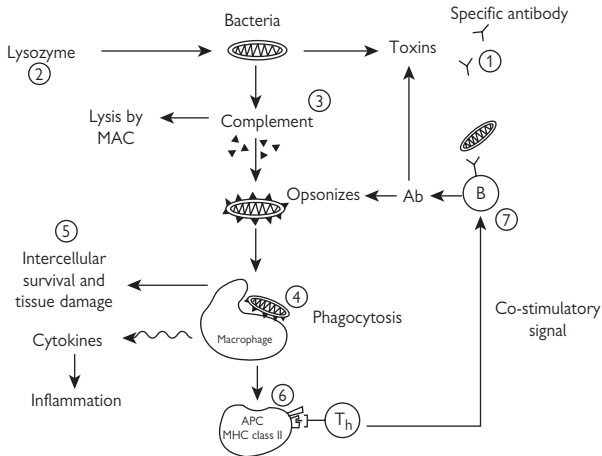


Fig. 1.3 Overview of bacterial defence.

Immunity against fungi

Superficial infections

The commonest fungal infections are usually superficial (e.g. the dermatophytes). In a healthy host these are controlled by constituents of sebaceous secretions, and cell-mediated immune responses (as demonstrated by the increased rate of severe skin/mucous membrane infections in those patients with AIDS).

Invasive infections

Other species can cause serious systemic infection, often entering the body as spores breathed into the lung. The manifestations of such infections depend on the nature of the immune response, and the immune avoidance mechanisms exhibited by the organism, e.g. *Histoplasma capsulatum* pulmonary infection may heal spontaneously, disseminate, or lead to the generation of chronic granulomas and fibrosis. This reflects the nature of the immune response and the organism's ability to resist digestion within macrophages. Host resistance may be weakened by specific immunodeficiency such as HIV infection or an inherited immunological defect. However, infections with organisms such as *Candida albicans* are significantly increased in those people with more general weakening in immune resistance caused by malnourishment, alcoholism, diabetes, iron deficiency, and age. The key players in the immune response to fungal infection include:

- antibody and complement responses – these opsonize fungal cells and facilitate phagocytosis (e.g. *Cryptococcus neoformans* possesses an anti-phagocytic capsule and can resist phagocytosis unless opsonized by antibody or complement). Antibody responses may also contribute to pathology (e.g. the hypersensitivity seen as part of infection with *Aspergillus* spp.)
- neutrophils – important phagocytic cells. Recurrent or severe fungal infections may be seen in those with defective neutrophil responses. They are a key part of first-line defence in organs such as the lung
- T-cells – severe infections of the skin and mucous membranes, as well as the lung and elsewhere are common in those with T-cell deficiencies (e.g. patients with AIDS). The response to chronic infection may lead to the development of granulomas.

Immunity against parasites

Protozoa

Protozoa owe their success as pathogens to a combination of strategies aimed at avoiding the full force of the immune response. These include adopting an intracellular habitat (e.g. toxoplasmosis), rapid antigenic variation (e.g. African trypanosomes use a gene-switching mechanism to repeatedly replace their outer coat as fast as antibodies are raised against it), immunosuppression (e.g. *Toxoplasma* suppresses T-cell function to facilitate its intracellular survival), and the generation of non-specific polyclonal B-cell responses which serve to inhibit the generation of effective specific immunity and may precipitate autoimmunity.

Worms

Eosinophils and immunoglobulin E (IgE) production are important players in the immune response to infection with worms, but as a result hypersensitivity reactions in the skin and lung may occur. It is unusual for worms to be eliminated by the immune response, and at most it serves to control their number. Eosinophilia results in part from mast cell and T-cell factors. Eosinophils phagocytose the antigen–antibody complexes that circulate in enormous quantities in worm infection, and modulate hypersensitivity. They may also kill certain worms. IgE production is stimulated by the presence of worms, and the resulting inflammatory response is thought to reduce worm attachment and gut entry. Chronic infection with certain flukes (e.g. schistosomes) can lead to fibrosis of the liver or bladder as a result of a T-cell-mediated reaction to their trapped eggs.

Basic principles of bacteriology

Taxonomy

Taxonomy is the art of dividing into ordered groups or categories. With respect to bacteria it refers to two main concepts:

- classification – the division of organisms into related groups based on similar characteristics. The species is the most definitive level of classification. Organisms may be reclassified from time to time as new information (e.g. genetic relatedness) becomes available
- nomenclature – the naming of groups and members of a group. This is governed by the International Committee on Systematics of Prokaryotes (www.the-icsp.org). The most recent revision of the International Code of Nomenclature of Bacteria was published in 1992. Amendments are published in journal form (the International Journal of Systematic and Evolutionary Microbiology). The basic rules for naming are outlined in Boxes 1.1 and 1.2.

Identification

Taxonomy is dynamic and throughout its history has been dependent on the techniques of identification available – originally phenotypic characteristics, and more-recently methods of determining the genetic ‘relatedness’ (phylogenetics) of a group of organisms, which should hopefully lead to a more-stable classification with fewer revisions in the future. Changes are overseen by the Judicial Commission of the International Union of Microbiological Societies.

- Phenotypic characteristics – cellular morphology, staining (e.g. Gram, or acid-fast, Box 1.3), motility, growth characteristics (speed, requirements, colonial appearance), biochemical characteristics (e.g. acid from specific carbohydrates), serology, analysis of metabolic end-products).
- Phylogenetic identification – nucleic acid hybridization (denaturation of double-stranded DNA into single strands and assessing their ability to anneal to the single strands of another related organism), 16S ribosomal ribonucleic acid (RNA) sequence analysis.

For identification purposes, simple phenotypic characteristics continue to be used which often (but not always) correlate with genotypes. These methods of phenotypic characterization have been developed and codified over years to facilitate laboratory identification of organisms (collected in texts such as *Bergey’s Manual of Systematic Bacteriology*).

Box 1.1 Microorganism nomenclature rules

- Each organism should have only one correct name. Where more than one exists, the oldest legitimate name takes precedence.
- Confusing names should be abandoned.
- Regardless of origin, all names are in Latin or are Latinized.
- The first word (genus) always starts with a capital letter.
- The second word (species) is in small letters.
- The genus and species name are underlined or italicized when printed.

Box 1.2 The naming hierarchy

- Order – names ending -ales
- Families – names ending -aceae
- Tribes – names ending -eae
- Genus
- Species – a collection of strains sharing common characteristics
- Strain – a bacterial culture derived from a pure isolate

Box 1.3 The Gram stain

Named after the Danish bacteriologist who devised it in 1844, the Gram stain remains a useful test – the outcome of which is determined largely by the structure of the cell wall. The procedure is simple:

- cells are stained with crystal violet
- then they are treated with iodine, forming a crystal violet/iodine complex in the cell
- next they are washed with an organic solvent (acetone-alcohol)
- then they are stained with a red counterstain, e.g. safranin
- Gram-positive organisms retain the crystal violet/iodine complex within the cell because of the thick peptidoglycan cell wall, and appear dark purple. In Gram-negative organisms the stain is leached from the cell due to disruption of the lipid-rich outer membrane by the organic solvent and they appear pink.

Structure and function of bacteria

Bacteria are prokaryotic – they have a single chromosome that is not enclosed in a nuclear membrane. They are around 0.2–2 micrometres by 1–6 micrometres long and exist in four basic shapes: cocci (spheres), bacilli (rods), spirillia (spirals), and vibrios (comma shaped).

Cytoplasm

Cytoplasm is a gel containing the enzymes, ions, subcellular organelles, and energy reserves of the organism. Energy and food are stored in membrane-bound granules. Glycogen is the major storage material of enteric bacteria. Ribosomes are the sites of protein synthesis. Bacterial ribosomes are 70S (the 'S' referring to a unit of sedimentation on ultracentrifugation) and are formed from 2 subunits – 30S (which contains 16S RNA) and 50S. Ribosomes are formed from specific ribosomal proteins and ribosomal RNA (they account for 80% of total cell RNA). They complex with a messenger RNA transcript from DNA to form polyribosomes (polysomes). Extrachromosomal DNA is often found within the cytoplasm in the form of plasmids. These are covalently closed double-stranded DNA (dsDNA) circles, and are capable of replication and are inherited by progeny cells. They may contain genetic information encoding structure or functions relating to bacterial virulence (antibiotic resistance, adhesions, toxins etc).

Cytoplasmic membrane

The cytoplasm is surrounded by the cytoplasmic membrane, a phospholipid bilayer into which various proteins are inserted. The membrane is involved in the synthesis and secretion of enzymes and toxins and active transportation of materials into the cytoplasm.

Bacterial cell wall

This provides rigidity and a physical barrier to the outside world. Peptidoglycan provides the strength and is found in all bacterial species except *Mycoplasma* and *Ureaplasma* spp. It comprises a carbohydrate backbone cross-linked by short peptides. Variations in the peptide linkages are responsible for different cell wall characters (see Box 1.3).

Gram-positive cell walls

These are composed of several layers of peptidoglycan, within which are trapped a variety of proteins, polysaccharides, and teichoic acids (polymers of glycerol or ribitol), which stabilize the cell wall and maintain its association with the cell membrane as well as having roles in cellular interaction and growth. They are antigenic in some organisms. Certain organisms will possess cell wall structures that confer virulence characteristics e.g. M protein of group A streptococci.

Gram-negative cell walls

These are thinner but more complex than those of Gram-positive organisms. Outside the cytoplasmic membrane is a periplasmic space. The outer part of this is bounded by a single peptidoglycan layer, beyond which lies the outer membrane – a phospholipid bilayer within which lie other large molecules. Lipoproteins link this membrane to the peptidoglycan below. Unique to gram-negative bacteria are the lipopolysaccharides (LPS) in the



outer membrane. These are the key surface antigens and endotoxins of Gram-negative organisms. They are composed of a lipid A (principally responsible for the endotoxin activity) attached to a core polysaccharide with side chains which vary within a species and confer the serological identity (O antigen) of individual strains. Other components of the outer membrane include: porin proteins (allow entry to the periplasmic space from the outside), and non-porin proteins (such as penicillin-binding proteins)

'Acid-fast' cell walls

Mycobacterium spp., *Nocardia* spp., and *Corynebacterium* spp. have a modified Gram-positive cell wall. They have a higher cell-wall lipid content which is due to mycolic acids, which can also confer virulence characteristics. Acid-fast organisms are so-called because once stained with red carbofuchsin dye they are resistant to decolourization with acid-alcohol – a property conferred by the cell wall lipids.

Bacterial surface structures

Capsules

Certain bacteria possess a capsule around their cell wall, usually composed of polysaccharide, but polypeptide in some organisms. They are manufactured at the cell membrane. They serve to protect cells from toxins, desiccation, complement proteins, and antibodies, and play a role in adherence (e.g. the glucan capsule of *Streptococcus mutans* forms the matrix of dental plaque). Capsules are antigenic and can be used to identify certain organisms (e.g. *Haemophilus influenzae* type B), and may be detectable in body fluids.

Flagellae

These are long thin appendages that are anchored in the cytoplasmic membrane and extend through the cell wall into the surrounding medium; they are responsible for cellular motility. They are usually found on Gram-negative rods, but motile Gram-positive organisms also exist. Flagellar number and arrangement vary from single (monotrichous) to multiple over the whole surface (peritrichous). The filament is composed of multiple flagellin proteins which have the capacity to self-assemble. The cell membrane-anchored base rotates as part of an energy-dependent reaction causing the rigid flagella to rotate. They are antigenic and several genera, e.g. *Salmonella* spp. are able to alter the antigenic type of flagella they produce (phase variation) by the differential expression of the genes coding various flagellin proteins.

Fimbriae

These are smaller appendages (~15–20 micrometres in length) composed of fibrillin and found on many Gram-negative bacteria. They form hollow tubes and are involved in attachment to cells or mucosal surfaces (also called adhesins). Different adhesins display different binding properties (e.g. mannose), which are partly responsible for the tissue tropism seen with certain species of bacteria. They are also involved in bacterial conjugation and the exchange of DNA from one cell to another. The term 'pili' is given to the fimbriae used by Gram-negative bacteria for DNA transfer in conjugation.

Bacterial genetics

Bacterial DNA

Bacterial genetic information is encoded in the cell's DNA, of which there are 2 types:

- chromosomal DNA – prokaryotic organisms have a single, covalently closed, circular chromosome of dsDNA. It lies in a supercoiled state within the cytoplasm, not enclosed but attached to the bacterial cell membrane at certain points. Individual genes are arranged linearly. In *E. coli* the chromosome contains around 5 million base pairs. DNA replication and transcription to mRNA occur continually (unlike eukaryotes)
- extra-chromosomal DNA – plasmids are small DNA molecules consisting of circular dsDNA. Replication is autonomous and occurs independently of the host cell. Multiple copies of the same plasmid and many different plasmids can coexist in the same cell. Plasmids pass to daughter cells and some are capable of transferring to other bacteria of the same (or other) species. They code for many different functions and structures, for example antibiotic resistance.

Genetic material can move between plasmids and from plasmid to chromosome (and vice versa) via transposons. These are DNA sequences that can copy themselves to new site, carrying associated genes with them.

In manufacturing proteins, single-stranded 'messenger' RNA (mRNA) is synthesized from dsDNA during transcription by a DNA-dependent RNA polymerase using the 'sense' strand of the DNA as a template. The mRNA forms a complex with several ribosomes (a polysome-mRNA complex). The mRNA is translated as transfer-RNA (tRNA) molecules bearing the appropriate amino acid, and its 'sense' bases associate with the mRNA 'anti-sense' bases.

Genetic variation

Genetic variation can occur by mutation or direct gene transfer.

Mutation

This occurs when one or more bases in the DNA sequence changes. It is permanent (barring remutation to the original sequence) and will be inherited by any progeny. Such changes may alter the amino acid sequence of the encoded protein or may change the circumstances in which a normal protein is produced (transcription changes). Mutations can be:

- deletion – losing a base will cause a frame-shift mutation, changing the amino-acids represented by the sequence from the point of mutation onwards. Deletions can involve several bases
- insertion – additional base or bases will also cause a frame-shift
- substitution – change of a single base to one of the other three changes the amino acid represented by the code.

Gene transfer

This is the main means by which bacteria achieve their rapid genetic variability. There are three mechanisms:

- transformation – the uptake of free bacterial DNA from the surrounding environment into recipient cells. Cells able to take up and

incorporate free DNA are termed 'competent'. This state is usually transient, occurring towards the late exponential phase of growth with the expression of surface receptors for DNA. DNA that enters can only be incorporated into the genome if there are homologous regions with which it can integrate (only DNA from related species is likely to achieve it) and requires the presence of the *recA* gene

- transduction – the exchange of genes by bacteriophages (or simply 'phages'). Phages are viruses that infect only bacteria. Certain phages integrate their genetic material into the bacterial host DNA. During phage replication, excision of viral sequence from the host DNA may result in fragments of bacterial DNA becoming enclosed within the viral particle. When this particle infects a new bacterial cell the DNA fragment recombines into the chromosome of the second bacterium. Transduction may be generalized (random accidental host DNA is transferred) or specialized (specific host genes are transferred as the phage DNA integrates at specific sites). Phage conversion refers to the phenomenon of phage DNA becoming integrated into the bacterial chromosome and bringing about a change in the bacterial phenotype – for example, toxin production in *Corynebacterium diphtheriae*. This is due to phage DNA precipitating the expression of otherwise unexpressed bacterial genes
- conjugation – the only mechanism that requires cell-to-cell interaction, and the major means by which bacteria acquire additional genes. Gram-negative cells achieve this by means of the sex pilus which is encoded on a specific plasmid (the F plasmid). The pilus establishes contact with another cell and is the tube through which DNA is passed. Some organisms integrate the F plasmid into chromosomal DNA – such cells are termed Hfr cells (high-frequency recombination). Gram-positive cells achieve conjugation by aggregating in response to the production of pheromones by the donor bacterium.

Bacterial growth and metabolism

Bacterial growth requires, logically enough, materials for the manufacture of cell components and a source of energy.

Materials

Some bacteria can synthesize all they require from simple raw materials. However, most pathogenic bacteria require a ready-made supply of the organic compounds they need for growth. Most of these nutrients diffuse freely across the cell membrane to enter the cell. Some are required at high concentration and uptake is energy dependent. Enzymes involved in these processes may be inducible (produced in the presence of the substrate) or constitutive (produced constantly and independent of the substrate).

Carbon

Lithotrophic bacteria are able to use carbon dioxide as the sole source of carbon, and use it as the basis of their organic metabolites. Thus the only other materials needed are water, inorganic salts, and energy. Organotrophic bacteria require organic carbon such as glucose – thus their energy source is also used in the synthesis of materials. Different bacterial species can utilize different organic carbon sources, *Pseudomonas* spp. being among the most versatile.

Nitrogen

Ammonium ions (NH_4^+) provide the nitrogen required by bacterial cells. This is turned into glutamate and glutamine, which in turn are processed into certain amino acids, purines etc. Certain bacterial species and blue-green algae can make ammonium direct from atmospheric nitrogen – predominantly soil-dwelling organisms, but certain human pathogens such as *Klebsiella* and *Clostridium* species can 'fix' nitrogen in this manner. Other organisms produce their ammonium ions by nitrate reduction or from the deamination of amino acids released from proteins.

Growth factors

Substances such as B vitamins, minerals, certain amino acids, purine, and pyrimidines are required by many bacteria, although not all are capable of synthesizing their own. An organism is described as prototrophic for a growth factor if it is capable of synthesizing it and does not require an exogenous source. All bacteria need certain inorganic ions such as magnesium and calcium, and some need zinc and copper among others.

Environmental conditions

As well as (of course) water and carbon dioxide, bacteria have specific optimal environmental requirements for growth, including temperature and pH. Oxygen requirements are discussed in the next section.

Energy

Bacterial metabolism is a balance between biosynthesis (anabolic) and degradation (catabolic reactions). Catabolic reactions power the biosynthetic processes as hydrolysis of substances being broken down liberates energy which is captured in the formation of the phosphate bonds of adenosine triphosphate (ATP).

An organism's ability to utilize certain carbohydrates (e.g. sucrose, mannose) and convert them to glucose (the starting point for both aerobic and anaerobic catabolism) for metabolism is a useful feature for characterizing bacteria. Many tests in clinical microbiology detect the acidic end-products of bacterial metabolism in controlled conditions.

The oxygen requirement of a specific organism reflects the means it uses to meet its energy needs.

- Obligate anaerobes – grow only in conditions of high reducing intensity, and oxygen is toxic.
- Aerotolerant anaerobes – anaerobic metabolism but not killed by the presence of oxygen.
- Facultative anaerobes – can grow in anaerobic and aerobic conditions.
- Obligate aerobes – need oxygen to grow.
- Microaerophilic organisms – best growth is seen in low oxygen levels; high levels may be inhibitory.
- Aerobes produce a free radical superoxide ($O_2^{\cdot-}$) which is reduced to oxygen and hydrogen peroxide. Catalase enzymes convert the latter to water and oxygen.

Anaerobic metabolism

Glucose use in anaerobic conditions is fermentation. This occurs via glycolysis, producing pyruvate and two molecules (net) of ATP per glucose molecule. Pyruvate can then enter several different pathways producing different end-products (e.g. lactic acid, acetaldehyde, ethanol, etc.)

Aerobic metabolism

Glucose use in aerobic conditions is respiration. Pyruvate forms in glycolysis as in anaerobic conditions but then enters the Krebs' cycle. Complete oxidation of glucose by these paths results in 38 molecules (net) of ATP per glucose molecule. The Krebs' cycle also produces precursors for several other important cellular components such as purines, pyrimidines, amino acids, and lipids.

Bacterial growth

Bacterial growth occurs as the mass of cellular constituents increases. Cell division starts once a critical mass is reached and occurs by binary fission. In a liquid medium, bacteria display a uniform growth curve.

- Lag phase – the cell synthesizes new enzymes and cofactors, and imports nutrients from the media.
- Increasing growth phase – enzymatic reaction rates approach steady state and cell growth begins.
- Logarithmic growth phase – cell growth and division is at maximum. This is influenced by temperature, the carbon source, oxygen, nutrient availability, and so on.
- Declining growth phase – nutrients are exhausted and growth slows.
- Stationary phase – new organisms produced equal those dying.
- Death phase – cells die off.

Bacterial virulence and pathogenicity

Definitions

Pathogenicity is defined as the ability of an organism to cause disease, whereas virulence is the the degree of pathogenicity within a group of organisms. Virulence is determined by several factors related to the organism and the host, most particularly the infectivity of the bacteria and the severity of the condition it produces. To be considered to be pathogenic, organisms will have strains of varying degrees of virulence.


Pathogenicity

Infection of the host is the necessary first step – infection does not, however, equate with disease. We are all colonized with many bacteria. These however only become disease causing in certain abnormal situations.

The organism must enter the host and attach to the mucous membrane surfaces. Some go no further than this and disease is caused by exotoxins (e.g. *Vibrio cholerae*), others penetrate deeper and multiply causing tissue damage and eventually gaining access to the blood and potentially disseminating. Some species such as mycobacteria are able to reside within cells, taking up a long-term residence within the host. Still others are highly specific in the organs they will infect (e.g. *Neisseria gonorrhoeae*). This may be related to the presence of specific receptors for bacterial attachment, or the presence of nutrients (e.g. *Brucella abortus* has a requirement for erythritol, which is found in the bovine placental tissue and results in localization of infection to this site).

Virulence factors

Adhesins

Bacterial cell surface adhesins adhere to complementary structures on the surface of susceptible cells. These adhesins may be fimbriae (see  Structure and function of bacteria, p.14), components of the bacterial capsule (see below), and other cell surface antigens. The adherence process is a prerequisite if a microorganism is to infect a cell.

Aggressins

These substances allow the cell to evade host defence mechanisms. These may act to prevent initial attack and phagocytosis or to enable to cell to survive once phagocytosed (with the added benefit that once settled within a phagocytic cell they are safe from continued exposure to antibody and complement). They include:

- **capsules** – enable organisms to avoid phagocytosis by preventing interaction with the bacterial cell surface and the phagocytic cell, or concealing surface antigens. Specific antibodies against the capsular material will opsonize the organism and allow its ingestion. Examples include *Streptococcus pneumoniae* and *Haemophilus influenzae* type B. Mycobacteria have components in their cell wall that prevent lysosome/phagosome fusion once ingested
- **extracellular slime substances** – these are surface proteins or carbohydrates (polysaccharides). Examples include the M protein of group A streptococci (*Streptococcus pyogenes*), which impairs complement function, protein A of *Staphylococcus aureus*, which binds

IgG by the Fc region, interfering with the phagocytosis of opsonized organisms, and the LPS of Gram-negative bacteria, which may delay or blunt the acute inflammatory response

- enzymes – some bacteria produce proteases which can hydrolyse and inactivate IgA, aiding mucosal colonization. *Listeria monocytogenes* secretes enzymes that inhibit destruction by the myeloperoxidase system of phagocytic cells. *Staphylococcus aureus* produces hyaluronidase, an enzyme that depolymerizes hyaluronic acid (responsible for cell-to-cell adhesion), thereby easing the spread of the organism. Group A streptococci produce streptokinases that lyse fibrin clots. *Clostridium perfringens* produces collagenases contributing to its ability to produce necrotizing skin infection
- siderophores – are molecules produced by most pathogenic bacteria and they scavenge iron from the host. Iron is required for virulence by several bacteria, and siderophores seem to protect bacteria from the killing effects of human serum
- plasmids – although these are not conventional virulence factors, they may code for a wide range of additional features promoting virulence such as antibiotic resistance, sex pili, and chromosomal mobilization (allowing the transfer of genetic material such as antibiotic resistance, toxin, etc to other cells).

Toxins

- Exotoxins are among the most potent biological toxins and are mainly produced by Gram-positive organisms. They are usually heat-labile proteins, and many can be inactivated by proteolytic enzymes or neutralized by specific antibodies. The effects are highly varied, e.g. the clinical manifestations of tetanus, botulism, and diphtheria are all due to toxins. Some toxins are secreted in the active form, whereas others require cleavage to become active, e.g. *Corynebacterium diphtheriae*¹.
- Endotoxins are only produced by Gram-negative bacteria and consist primarily of LPS. They are heat stable and are only partly neutralized by specific antibodies. They are relatively low toxicity compared to exotoxins. They cause fever, hypotension, haemorrhage, and disseminated intravascular coagulation, and stimulate cytokine release from macrophages.

¹ Interestingly only those strains that contain a lysogenic bacteriophage (β -corynebophage) are able to produce the toxin. The toxin genes exist on the phage genome.

Basic principles of virology

Classification

Viruses are classified on the basis of a number of criteria. These include:

- type of nucleic acid (DNA, RNA) and strand number (single, double)
- conformation of the nuclear material (linear, circular etc)
- whether the genetic information is positive or negative sense
- nucleocapsid symmetry
- presence or absence of an envelope
- their antigenic or genetic similarity.

They are grouped into orders, families, subfamilies, and genera. There are no consistent rules governing the naming of individual viruses. Some are named according to the disease they produce (poxvirus), others by acronyms (papovavirus – papilloma polyme vacuolating virus), some by appearance (coronavirus), and still others after the location in which they were first identified (Marburg). They may rarely be called after their discoverers (Epstein–Barr). Official names should be Latinized and printed in italic.

Properties and structure

Viruses are small (20–150 nm in diameter) protein packages, containing genetic material (DNA or RNA); some also contain enzymes. Viruses depend on living cells for their existence, genome expression, and replication. They have colonized most life forms including bacteria, plants, insects, and animals. The viral particle is composed of structural proteins. A capsid (protein coat) protects the nucleic acid contents and facilitates viral entry to a host cell. It is composed of many capsomeres (protein subunits). The term nucleocapsid refers to the capsid and viral nucleic acid. Certain viruses contain enzymes (e.g. reverse transcriptase in HIV). Some viral capsids have an outer envelope (derived from the plasma membrane of the infected cell from which it was released), into which are embedded protein spikes. Beneath the envelope, some viruses may have a stabilizing membrane protein. The entire particle is referred to as a virion.

Nucleocapsids may take several geometric forms:

- helical (like a spiral staircase) – the nucleic acid forms the central core with the nucleocapsid proteins forming the steps e.g. ssRNA viruses such as influenza and rabies. These viruses are enveloped, the envelope itself resting on the underlying membrane protein shell
- icosahedral (20 triangular faces with 12 corners) – e.g. all human DNA viruses. Each capsomere may itself be made of several peptides. DNA-herpesviruses have icosahedral structure and are additionally surrounded by a lipid envelope
- complex – these viruses do not fall into neat structural categories and often have large genomes e.g. poxvirus.

Viral genomes

The genetic material may be DNA or RNA (RNA viruses tend to have smaller genomes). Nucleic acid conformation varies widely between viral families (double-stranded, single-stranded, linear, circular, etc). Genomes vary widely in size but are limited by the space available in the virion. Bacteria may have several thousand genes but even the largest viruses have less than 200 and the smallest perhaps only four (such viruses may produce



more than one protein from the same gene by means of RNA splicing or frame shifting). Viruses evolve rapidly due to the high number of genome duplications undergone in short spaces of time. RNA viruses have high error rates, with genomes diverging by as much as 2% in the course of a year – 1 million times the rate of eukaryotic cell DNA genomes. Many mutations are non-functional but some will allow the virus to evade host immune responses and medical therapies.

The genome encodes both structural and non-structural (NS) proteins (enzymes required for viral expression and replication). The manner in which expression occurs depends on the nature of the nucleic acid:

- DNA viruses make RNA copies of the relevant segments of their DNA to direct protein synthesis – they may use host enzymes to achieve this or rarely carry them within the virion
- positive sense RNA viruses produce messenger RNA directly
- negative sense RNA viruses possess enzymes that produce positive strand copies that are used as mRNA
- retroviruses produce DNA from their RNA. This is integrated into the host's chromosomal DNA, transcription then taking place in broadly the same way as host mRNA is made from host DNA.

Viral replication

The manner in which a virus infects a cell varies but generally involves the interaction of a viral protein and host cell receptor (proteins, glycoproteins, or glycolipids intended for other functions and simply exploited by the virus), precipitating internalization. Once in the cell the virus uncoats (sheds its protein shell) and frees the nucleic acid, at least partially. It needs to achieve two things: the production of its enzymes and structural proteins; and replication of the viral genome.

Transcription

The manner in which mRNA is produced depends on the nature of the genome (see above). Ribosomes translate the viral mRNAs. Proteins may be produced in phases: 'early' proteins may be involved in DNA synthesis, or act as transcriptional activators to speed viral expression over host proteins. 'Late' proteins are produced from mRNA transcribed from newly synthesized viral nucleic acid, and tend to be structural. Some viruses produce a single long polypeptide which is then cleaved by proteases into individual proteins. Proteins often undergo post-translational processes such as glycosylation.

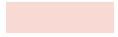
Viral genome replication

RNA viruses produce an RNA polymerase, either packaged with the virion (negative-sense viruses) or manufactured upon infection (positive-sense viruses). This rapidly produces RNA copies for incorporation into the viral particles. Genetic variation may arise by two mechanisms:

- mutations can occur due to errors in replication and the absence of proof-reading activity in enzymes such as RNA replicase and reverse transcriptase. DNA viruses replicate their genomes in the host nucleus where the necessary host enzymes can be exploited. The exceptions are the poxviruses which carry DNA polymerases with them and are thus capable of working entirely in the cytoplasm
- recombination of genetic material can occur either within a genome or between two viruses of the same kind if the host cell is co-infected with both viruses. RNA viruses are also capable of gene reassortment which, although not true mutation, may result in progeny with a quite different phenotype from parental strains, e.g. influenza pandemics may be a consequence of reassortment between human, avian, and pig flu.

Viral assembly

This may occur predominantly in the nucleus (e.g. adenovirus), or in the cytoplasm (e.g. poliovirus). Viral release then occurs by budding from the cell surface (e.g. measles), lysis of the cell (e.g. polio), or cell-to-cell spread. Some, such as HIV, may require a phase of post-release maturation. Overall, a complete viral lifecycle typically takes 6–8 hours, with the potential to produce thousand of viruses from each infected cell.



Viral pathogenesis

The effect of viral infection on the host ranges from asymptomatic infection to devastating disease with a wide variety of clinical manifestations. Viral species vary in pathogenicity and different strains of the same species may vary in virulence.

Entry

Viruses enter the body through the skin (usually via some degree of trauma) or via mucous membranes (where they adsorb directly to epithelial cells, in which they undergo primary replication). Resulting infections may be localized (e.g. papillomavirus and warts, conjunctivitis) or generalized, in which case pathology is not necessarily focused at the organ initially infected (e.g. enteroviruses are spread faeco-orally yet cause encephalitis). Transmission may also occur vertically (mother to child) and iatrogenically (organ transplants, blood transfusions etc).

Cytopathic effect

Viruses may disrupt the function of the cells they infect. This may result in inhibition of host-cell protein manufacture and lead to the death of the cell, lysis, and the release of virion. Alternatively they may precipitate cell fusion, forming multinucleated giant cells, or syncytia (e.g. respiratory syncytial virus (RSV)). Others may form inclusion bodies within the cell (eosinophilic or basophilic staining areas within the cell, representing aggregations of virions, sites of viral synthesis, or degenerative change). These inclusions can occur within the cytoplasm or nucleus.

Extent of infection

Some viral infections remain confined to tissues at, or continuous with, the site of entry. They may form focal lesions (e.g. skin and papillomavirus) or affect large areas of specific mucous membranes (e.g. viral gastroenteritis), and tend to have short incubation periods. Other viruses produce generalized infections, an initial phase of local replication near to the site of entry being followed by haematogenous spread (primary viraemia) to regional lymph nodes. This allows infection of large reticuloendothelial organs resulting in a secondary viraemia. The virus may travel free in the blood or within infected blood cells. It travels to organs distant from the site of entry and may infect specific organs preferentially. Such infections tend to have longer incubation periods.

Target organs

Symptoms depend on the target organ:

- skin – rashes are a common feature of viral infections and may be due to virus replication in the skin (vesicular rashes of Herpes simplex and Varicella zoster), the killing of infected cells (measles) or more general features of infection (disseminated intravascular coagulation (DIC), thrombocytopenia)
- respiratory tract – the lung may be involved in local infections (e.g. influenza) but can also be involved in generalized viral infections (e.g. chickenpox or measles pneumonitis)

- liver – the hepatitis viruses (hepatitis A to E) are tropic for the liver, which may also be affected as part of a more generalized infection (e.g. Epstein–Barr virus (EBV), cytomegalovirus)
- central nervous system (CNS) – the CNS can be invaded either as a result of viral passage along nerves (e.g. rabies) or by haematogenous spread (e.g. polio).

Illness duration

Viral illness may be acute or chronic.

- Acute viral illnesses present within a relatively short period of time. Most such infections are mild with a quick spontaneous recovery, e.g. chickenpox, measles, mumps, rubella. Some may have delayed serious features after an apparent recovery (e.g. encephalitis), or cause a rapid decline and possible death (e.g. rabies, viral haemorrhagic fevers).
- Chronic or persistent infections require the survival of viral DNA within the host cell, either integrated within the host DNA or in episomal form separate from it. They may be latent with no apparent illness or virus but occasional periods of reactivation (e.g. herpes simplex, varicella zoster), or chronic with continuous production of infectious virus (hepatitis B, hepatitis C, HIV).

Transformation

Some viruses have the potential to induce malignant change. For example EBV is associated with Burkitt's lymphoma, nasopharyngeal carcinoma, primary cerebral lymphoma and post-transplant lymphoproliferative disorder (PTLD).

