Chapter 1

Introduction to medical microbiology

This chapter considers medical microbiology from the points of view of its nature, history, the host–parasite relationship and lastly epidemiology.

**NATURE OF MICROBIOLOGY**

Microbiology is the study of the organisms or microbes that cause infection. Microorganisms may be eukaryotic, prokaryotic or subcellular.

**Eukaryotes**: uni- or multicellular organisms that have

- A nucleus surrounded by a nuclear membrane – contains the genomic DNA in the form of several chromosomes.
- Membrane-bound organelles – such as mitochondria.
- Biochemistry differing from that of prokaryotes

**Prokaryotes**: unicellular organisms that have

- No nuclear membrane – no distinct nucleus, the genomic DNA lies free in the cytoplasm.
- No membrane-bound organelles
- Biochemistry differing from that of eukaryotes
- Size – about 1000nm, much smaller than eukaryotic cells.

**Types of microorganism**

**Bacteria**: prokaryotes with a rigid peptidoglycan cell wall; divide by binary fission.

**Viruses**: not cells and smaller than bacteria, contain either DNA or RNA as the genome; metabolically inert but capable of replication by taking over the synthetic machinery of a susceptible cell.

**Prions**: not cells, are a conformationally altered form (PrP\text{Sc}) of a normal cellular protein – PrP\text{C}. Contain no detectable nucleic acid.

**Fungi**: eukaryotic, with a rigid cell wall; unicellular, round yeast-like forms or filamentous branching forms bearing conidia or spore-like structures.

**Parasites**: eukaryotic, either unicellular protozoa, or multicellular helminths (worms); replication often complex, especially with helminths, the life cycle often involving intermediate animal hosts.

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Since Biblical times it has been known that some diseases can spread from person to person, i.e. that they are infectious. However, it was millennia later before the causes were identified. Some pioneers in microbiology:

**Antony van Leeuwenhoek**: a Dutch draper who built a microscope and in 1675 observed ‘animalculi’ (now known to be microorganisms) in water, soil and human material.

**Edward Jenner**: showed in 1796 that smallpox could be prevented by inoculation with the related disease cowpox, although he did not discover the nature of the infectious agents involved.

**Louis Pasteur**: the founder of modern microbiology; from 1867 to 1888 developed methods for culturing bacteria, and propagated the virus of rabies in animals; he also established the principles of the preparation of vaccines against infectious diseases.

**Robert Koch**: a German bacteriologist, discovered and cultured the bacteria that caused several diseases, including tuberculosis, during the latter part of the 19th century; he used solidifying agents such as agar – allegedly on the advice of Frau Hesse, the wife of a colleague – to prepare solid media on which bacteria could be grown in individual colonies and therefore in pure culture. He laid down the famous criteria for confirming an organism as the cause of a specific disease, i.e. **Koch’s postulates**:

1. It must be found in all cases of the disease and its distribution must correspond to that of the lesions observed.
2. Able to be cultured outside the body for several generations.
3. Should reproduce the disease on inoculation into susceptible animals.

Nowadays a fourth postulate would be added:
4. Antibody to the organism develops during the course of the infection.

**Note** – many infectious diseases of which the causal organism is clearly identified do not fulfil the third and fourth, nor even occasionally the second, postulates.

**Joseph Lister**: Professor of Surgery in Glasgow Royal Infirmary, applied Pasteur’s observations to show in 1867 that wound sepsis could be prevented by using an antiseptic technique with carbolic acid.

**Dmitri Ivanovski**: in 1892 discovered and studied tobacco mosaic virus in plants and differentiated it from bacteria by showing that it passed through filters that retained bacteria which are larger.

**John Enders, Thomas Weller and Frederick Robbins**: showed in 1949 that poliovirus could be propagated in monkey kidney cell cultures, and thus founded the study of modern virology – and the development of polio and other virus vaccines.
THE HOST–PARASITE RELATIONSHIP

The relationship between the host and the parasite (the infecting organism) determines the outcome of an infection. However, many organisms can coexist without ill-effects on the host – usually as part of the body’s normal flora.

THE HOST

Apart from general physical and chemical defences, the host defends itself against infection using its immune system:

**Innate (or natural) immunity:** not directed at a particular organism. Mediated by complement, phagocytes, natural killer (NK) cells, eosinophils and basophils together with cytokines released from infected cells. The cells and molecules of the innate system work in concert to produce the acute inflammatory response.

**Adaptive (or acquired) immunity:** directed at a particular organism. The adaptive response operates in two main ways

- **Humoral** – due to specific antibody production by B lymphocytes (B cells).
- **Cell-mediated** – due to T lymphocytes (T cells) and the cytokines they produce.

The various defence mechanisms differ in their effectiveness against bacteria and viruses.

Physical and chemical defence mechanisms

**Skin:** a tough and impermeable barrier unless breached.

**Normal flora (‘commensals’):** the presence of harmless bacteria in various body sites in the normal healthy individual can make it difficult for exogenous pathogens to invade and establish themselves.

**Flushing effect:** tears, the flow of urine and the upward flow of mucus by ciliated respiratory epithelium all act to prevent attachment and remove invading organisms – both bacteria and viruses.

**Gastrointestinal tract:** the low pH of stomach acid helps to inactivate acid-labile viruses and, less effectively, ingested bacteria.

**Vaginal secretions:** in young women these have a low pH due to lactobacilli in the normal flora, and so have a protective effect against bacterial infection.

**Innate immunity**

**Phagocytosis:** an important defence mechanism whereby both bacteria and viruses are ingested by two types of scavenging cell

- **Neutrophil polymorphonuclear leucocytes** – ‘neutrophils’.
- **Macrophages** – mononuclear phagocytes. There are two types of these cells
  - Free macrophages in lung alveoli and the peritoneal cavity.
  - Fixed macrophages in lymph nodes, spleen, liver (Kupffer cells), connective tissue (histiocytes) and brain (microglia).
Phagocytosis is enhanced by antibody (a specific immune mechanism) and complement: this effect is known as opsonization; macrophages ‘activated’ by cytokines released by T lymphocytes (also a specific immune mechanism) have increased phagocytic activity and are attracted by chemotaxis to the site of infection.

NK cells: large granular lymphocytes lacking the antigen specific receptors found on T and B lymphocytes. Kill infected cells by inducing apoptosis (programmed cell death).

Complement: a set of plasma proteins which upon infection become activated in a cascade to enhance phagocytosis, lyse microorganisms or infected cells and increase vascular permeability.

Cytokines: small protein molecules released by many cells, including lymphocytes and macrophages, which function as signals or mediators to activate, modulate and control the immune responses and other activities (such as tissue repair, differentiation, and signalling activity in the central nervous system – CNS) of various cells. There are numerous cytokines, for example interleukins, tumour necrosis factor (TNF) and interferon which has important antiviral activity (see Chapter 21). Among other functions, cytokines
- Inhibit immune cell migration
- Attract lymphocytes, macrophages and polymorphonuclear leucocytes – to the site of infection by chemotaxis, also known as chemokines
- Increase capillary permeability
- Induce mitogenic activity – by stimulating the transformation of lymphocytes
- Help in the production of IgE – the antibody responsible for allergic reactions; results in mast cell activation.

Adaptive immunity

Humoral (antibody) response: antibodies are immunoglobulins (proteins which react with specific antigens on the surface of organisms or cells) found in the blood and other body secretions. Antibodies opsonize or coat bacteria to prepare them for phagocytosis and lysis, neutralize viruses and are responsible for specific and long-term immunity to infection; they are produced by B (bone marrow-derived) lymphocytes
- B lymphocytes – cells with immunoglobulin on their surface which acts as the receptor for antigen on the invading organism. Encounter of B lymphocytes with antigen in lymphoid tissue (e.g. spleen, lymph node) activates the lymphocytes causing them to divide and develop into antibody-secreting plasma cells. Antigen is presented by macrophages, dendritic cells and B cells and the involvement of T lymphocytes is required to initiate the immune response to most microbial antigens.
- Immunoglobulins – proteins composed of polypeptide chains: the Fc fragment activates complement and binds to receptors on infected host cells; the two Fab fragments contain the antigen-binding sites.
Five immunoglobulins are responsible for humoral immunity

- IgM, as a monomer on the B cell membrane, the main receptor for antigen; as the secreted form a pentamer of five immunoglobulin subunits, the earliest antibody produced appearing in the blood soon after infection, persists for a few weeks and protects mainly against blood-borne organisms.
- IgG, produced in the blood later than IgM and conveys long-term immunity, especially to viruses – protects the tissues and is the only antibody to cross the placenta to protect the unborn child.
- IgA, a dimeric molecule found in body secretions such as saliva, respiratory secretion, tears and gastrointestinal contents (as well as blood); the main antibody involved in immunity to respiratory and gut infections; ‘secretory’ IgA, acquires a carbohydrate transport piece in extracellular fluids that is absent from serum IgA.
- IgE, activates mast cells and basophils. Important role in allergic reactions, and raised in certain parasitic infections.
- IgD, mainly an antigen receptor that regulates B lymphocyte activation.

Cell-mediated immune response: mediated by T (thymus-derived) lymphocytes and includes the release of cytokines. Two main cell types are involved.

- Helper T cells – have CD4 molecules on their surface; require major histocompatibility complex (MHC) class II molecules on macrophages or dendritic cells (found in lymph nodes and skin) to present the target antigen for their activation; interact with B lymphocytes to induce antibody production
- Cytotoxic T cells – have CD8 molecules on their surface; require MHC class I molecules to present the target antigen for their activation; kill target cells, such as virus-infected cells, by the same mechanism as NK cells, i.e. induce apoptosis.

PORTAL OF ENTRY

Microorganisms enter the body in six main ways

- Respiratory tract – via inhalation.
- Alimentary tract – by ingestion.
- Genital tract – via sexual intercourse.
- Skin – via abrasions, via the bite of an arthropod or other animal.
- Other – conjunctiva, blood transfusion, injections (e.g. medically administered or via shared syringes in drug abuse), organ transplants.
- Congenital – or vertically, from mother to fetus.

PATHOGENIC MECHANISMS OF THE PARASITE

Infecting organisms vary in their pathogenicity or ability to produce disease. Virulence is a commonly used but ill-defined term which indicates the degree of pathogenicity. Opportunistic infections are caused by organisms of low virulence where host resistance is reduced.
Bacteria

*Normal flora (‘commensals’):* bacteria usually found in the normal healthy individual but without harm and only occasionally cause disease, for example in the immunocompromised.

*Invasiveness:* the ability to spread within the body of the host, principally due to toxin production; other properties, such as the possession of a capsule, may enable the bacterium to evade defence mechanisms such as phagocytosis.

*Toxin production:* bacteria produce two types (Table 1.1)
- *Exotoxins* – secreted by bacteria.
- *Endotoxins* – an integral part of the bacterial cell wall (see Chapter 2) and usually only released when the bacterial cell dies.

*Immunopathology:* in some bacterial infections the host immune response contributes to pathogenesis.

Viruses

Viruses are important and common causes of human disease, especially in children. Most infections are mild and the patient makes a complete recovery; many are silent and the virus multiplies in the body without causing any symptoms at all. However, viral infections which are usually mild sometimes cause severe disease in an unusually susceptible patient. A few viral diseases are severe and always have a high case fatality rate.

*Invasiveness:* viruses replicate within cells by taking over their metabolic activities and redirecting them to the synthesis of viral components, with subsequent assembly into new infectious virus particles; this usually (but not always) kills the cell and thus produces lesions and disease within the tissue or organ to which the cell belongs.

*Immunopathology:* in some virus infections the host immune response contributes to pathogenesis.

*Oncogenesis:* some cancers or neoplasias have a viral aetiology.

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<th>Table 1.1 Bacterial toxins</th>
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<tr>
<td><strong>Composition</strong></td>
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<td>Action</td>
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*Toxoid is toxin treated, usually with formaldehyde, so that it loses toxicity but retains antigenicity.

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Prions

Prions produce disease by accumulation of PrPSc (a conformationally altered form of a normal cell protein) in neural cells disrupting function and causing vacuolization and cell death.

Fungi

*Invasiveness:* fungi multiply in mucosal tissues and keratin, producing common superficial infections; fungi which have gained access to tissues cause subcutaneous infections; in the bloodstream they cause life-threatening systemic infections, especially in immunocompromised patients.

*Toxin production:* ingestion of mouldy food in which fungal metabolites have been produced causes serious food poisoning.

*Immunopathology:* inhalation of fungal hyphae or spores causes host hypersensitivity.

Parasites

Parasites are much more complex than bacteria or viruses and cause disease by many different routes.

**NATURE OF EPIDEMIOLOGY**

Microbiology includes epidemiology which is the study of the spread of infection, usually exogenously acquired, within a community or the population at large.

**EPIDEMIOLOGY**

Spread of an organism in a population depends on:

*Reservoir of infection:* where the infectious agent is normally found and where it may multiply or survive; may be human, animal – a *zoonosis* is an infectious disease of non-human vertebrates that can be transmitted to humans; or the inanimate environment.

*Source of infection:* may be patient’s own microflora (endogenous) or another human being, or an animal (zoonosis) or an environmental source (exogenous). When the source of infection is inanimate, e.g. food, water, soil or fomites (see below), the latter constitutes the vehicle of infection.

*Note* – source and reservoir may be the same or may differ, e.g. the reservoir of hepatitis A is humans but infected faeces contaminating drinking water may be the source of an outbreak.

*Route of transmission:* by inhalation of droplets, droplet nuclei (smaller than droplets), aerosols or spores, ingestion (faecal–oral or faeces-to-hand-to-mouth), percutaneous, sexual, transplacental (mother to child). The terms *nosocomial* and *iatrogenic* are used for infections acquired in hospital and those related to medical treatment, respectively. May be direct or indirect.
Direct spread: from person to person by physical contact or inhalation.
Indirect spread: via fomites, inanimate objects, e.g. door handles, bed clothes, that may be contaminated with infectious agents, or via food and water.
Vectors: many infections are spread by biting arthropods, a common route in tropical countries.
Herd immunity: generally dependent on the level of protective antibody in the population; when this is low the organism can find many susceptible hosts to infect and vice versa.

Measurements in epidemiology

Infectious disease is continuously monitored by epidemiologists and microbiologists; although infection cannot always be halted, prompt preventive measures may succeed in controlling an outbreak. Below are some of the measurements used in the surveillance of infectious disease.

Incubation period: the period from the start of infection to the initial appearance of the characteristic symptoms of the disease; very variable, and in some diseases impossible to measure, but important to determine in order to identify sources of infection in outbreaks.

Period of communicability: the time (days, weeks, or months) during which an infectious agent may be transmitted, directly or indirectly, from an infected person to another person; from an infected animal to humans; or from an infected person to animals or arthropods. Knowledge of this is important for infection control.

Incidence: of infection or disease is the proportion of a population contracting that infection or disease during a specified period, usually a year; expressed as a ratio, e.g. per 1000, or per 100,000 in the population concerned.*

Prevalence: refers to the proportion of a population infected (or sick or immune) at a specified point in time. Can be used only for states of relatively long duration, i.e. immunity, persisting infection or chronic disease.*

Attack rate: the incidence of infection in a defined group, e.g. the inhabitants of an institution.

Secondary attack rate: the number of cases appearing in contacts of the first or index case.

Mortality rate: expressed as the ratio of number of deaths from a disease in a given year to total population at mid year.

Case fatality rate: the proportion of patients with the disease who die from it.

Epidemics

These may be confined to one country or region but may also be seen worldwide, when they are known as pandemics; the AIDS epidemic is an example of the latter. Most are viral but some, such as cholera, and plague in former centuries, are bacterial.

* e.g. influenza – incidence of disease and prevalence of immunity.

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Endemic infection: the constant presence of the infectious disease or its agent within a population.

Epidemics: recognized when the number of cases of infection rises above the expected level, or when a new infectious agent appears within the community concerned; the difference between epidemics and outbreaks – the latter implying a lower number of cases than in an epidemic – can be hard to determine.

Pandemic: a worldwide epidemic.

Note – cases may be sporadic, i.e. not known to be related to each other, or clustered, i.e. two or more related cases of infection suggesting the possibility of a common source or transmission between cases.

Bacterial epidemics

Rarer than viral epidemics but still important. The most famous epidemic in history was the Black Death, i.e. bubonic plague, which spread from China through Europe in the 14th century, killing around one-third of the population. Nowadays cholera is in its seventh pandemic, which started in 1961; tuberculosis in human immunodeficiency virus (HIV)-infected persons is at present epidemic in Africa. Dysentery, salmonella and campylobacter food-poisoning, and meticillin (formerly methicillin) resistant Staphylococcus aureus (MRSA) in hospitals are examples of bacterial diseases that remain out of control in many parts of the world, including developed western countries.

Viral epidemics

Many of these have been controlled or even eliminated by vaccination. HIV and its end-stage infection as AIDS is currently the most important infectious disease problem facing the world. Other epidemic virus diseases include influenza – which despite an available vaccine kills many thousands of patients in an epidemic year – and several of the vector or arthropod-borne infections, such as dengue and West Nile fever, which have both in the last few years spread beyond their traditional boundaries.

IMMUNIZATION

Without doubt, the advent of successful vaccines has had the most dramatic effect on human health of anything in medicine. Immunization has controlled diphtheria, tetanus, measles, mumps, rubella, yellow fever and, to a lesser extent, hepatitis B. Vaccination, together with appropriate control measures, succeeded in eliminating smallpox from the world altogether. Poliomyelitis is likely to follow soon.