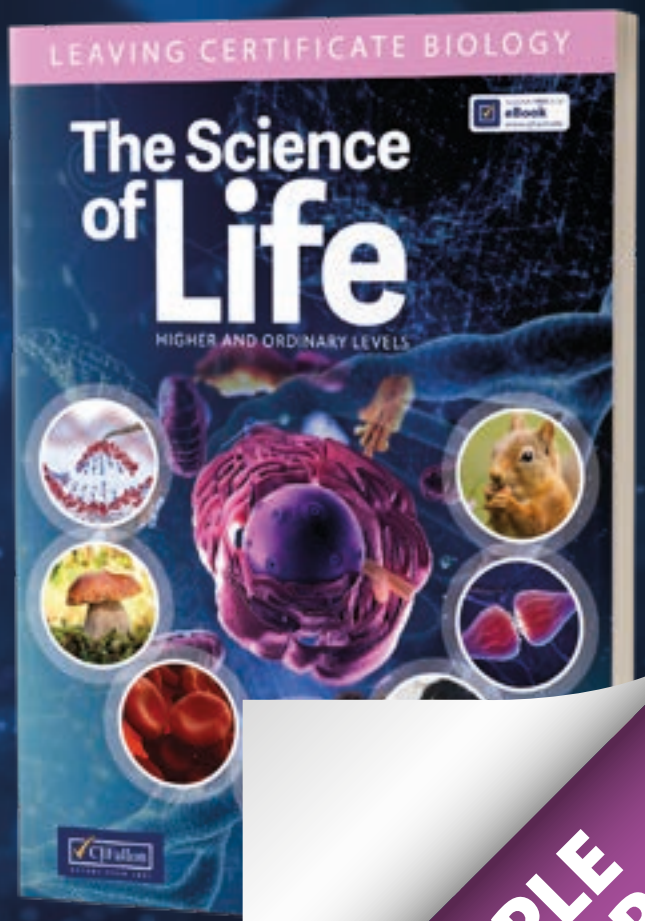
BIOLOGY FOR LEAVING CERTIFICATE

The Science of Life

The Science of Life is a concise, up-to-date textbook and accompanying Research and Investigations Book for the **NEW Leaving Certificate** Biology course. Written by two highly experienced Biology teachers and authors, this textbook aims to instil curiosity in the living world, while also supporting students through Biology at both Ordinary and Higher Levels.

It provides students with all the resources and opportunities they need to:

- Understand the core biological concepts, which are explained using clear concise language.
- Apply their knowledge and skills to solve problems.
- Develop models to represent structure, function and interactions in biology.
- Learn an evidence-based habit of mind through scientific inquiry.
- Safely use the materials and equipment of modern practical biology.
- Practise the skills they need to plan and carry out investigations in the laboratory and in the field.
- Research the important ways biological knowledge is applied in industry, agriculture and medicine.
- Appreciate and evaluate the impact of technological advances in biology on society.
- Develop a critical awareness of the impact of human activity on biodiversity and ecosystems.
- Explore ways in which biology can provide solutions to the climate and biodiversity crisis.



SAMPLE CHAPTERS

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Contents

CHAPTER 12

Immunity and Disease

At the end of this chapter you should be able to:

- Recognise the range of pathogens and the diseases they cause.
- Explain the concept of immunity.
- Distinguish between innate and acquired immunity.
- Outline the range of responses that are part of the innate immune response.
- HL Outline the role of monocytes, and how they become macrophages that link the innate and adaptive immune response.
- HL Distinguish between antigens and antibodies and describe how they interact during the immune response.
- HL Describe the activity and interaction of lymphocytes in the adaptive immune response.
- HL Compare the roles of different types of white blood cells in the immune response.
- Explain how immunity may be acquired actively or passively.
- Distinguish between artificial and naturally acquired immunity.
- Examine the interrelated factors that contribute to the emergence of infectious disease in plants and animals.
- Outline how the spread of disease is dependent on various interrelated factors.
- Outline the strategies applied to prevent and treat microbial diseases.
- Recognise how local, regional and global movement of organisms facilitates the transmission and spread of disease.
- Discuss the importance of a knowledge of emerging diseases in society.
- List the factors leading to increased numbers of cases of autoimmune diseases.
- Outline strategies to control the increase in cases of autoimmune diseases.

To help you understand this chapter, remember:

- The characteristics of different types of biomolecules (Chapter 3).
- The structure and function of the components of the cell membrane (Chapters 3 and 4).
- The role of proteins as receptors and in signalling (Chapter 3).
- The structure and function of viruses as obligate parasites (Chapter 2).
- How mutations arise and lead to variation (Chapters 6 and 10).
- Antibiotic resistance arising through evolution by means of natural selection (Chapter 6).

In this chapter you will learn how the body defends itself against pathogens, distinguishing between the innate and adaptive immune systems. You will learn about the role of antibodies and the action of various specialised white blood cells in immunity. Through your exploration of the emergence and spread of infectious diseases, you will evaluate strategies to prevent and control their transmission in populations.

Disease and Immunity

Disease

There are many ways in which the normal functioning of an organism can go wrong and cause harm, a condition we call **disease**. Diseases can be infectious or non-infectious.

Infectious diseases are caused by some kinds of organisms that can invade our body and cause illness. Not all infectious diseases are contagious, however. Contagious diseases are those that can spread from one person to another.

Definition: **Infectious diseases** are caused by certain organisms that can invade our body and cause illness.

Definition: **Infection** is the invasion of an organism by a pathogen.

Definition: A **pathogen** is an organism that causes disease.

Definition: A **parasite** is an organism that benefits by living in or on another organism (called a host), causing it harm.

Pathogens and Parasites

Organisms that cause disease are called **pathogens**. Most pathogens are microscopic (bacteria, fungi, protists and viruses), but some macroscopic **parasitic animals** such as worms can also cause infectious disease (Fig. 12.1).

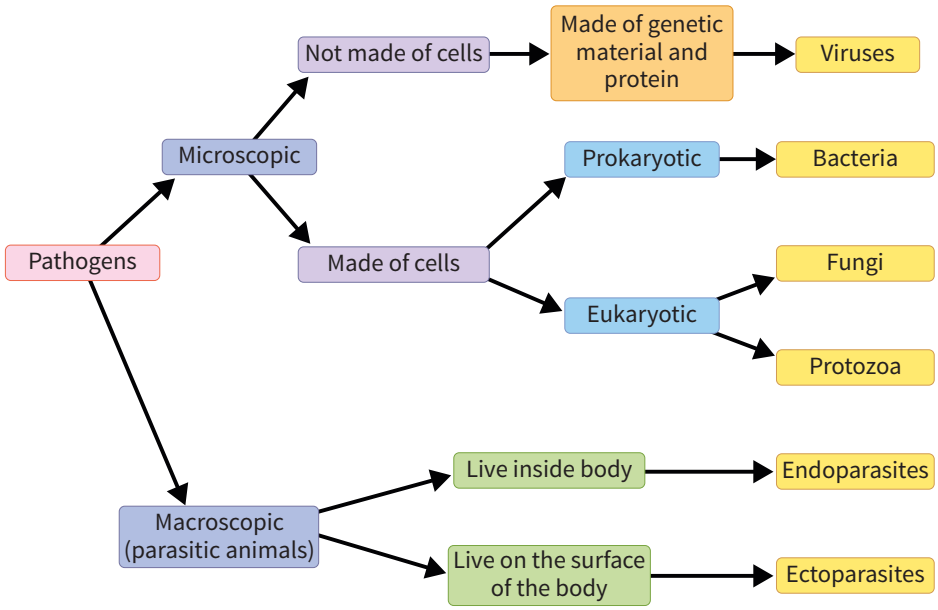


Fig. 12.1 *(A) Tapeworms and (B) ticks are examples of parasites that cause harm.*

Parasites are organisms that gain benefit from another organism, causing it harm. All **parasites** are pathogens, because they cause harm (disease). Some human parasites are animals such as worms, ticks, fleas, lice, etc. A number of members of the Kingdom Protista cause devastating infectious disease. Malaria is caused by a unicellular protist which is transmitted by the mosquito. Protist parasites also cause sleeping sickness and cryptosporidiosis.

All **viruses** and many pathogenic bacteria are **obligate parasites**. This means that they cannot survive and reproduce (or replicate) without a **host** (the organism the parasite harms and gains benefit from).

Extend Your Knowledge

Scan this code to read an article about tapeworms.

A **prion** is a type of protein that can trigger normal proteins in the brain to fold abnormally. It is a very unusual pathogen because, unlike bacteria or viruses, it does not contain any DNA or RNA but it can still replicate. Prions can affect humans and other animals. They can be spread to humans who eat meat from an animal infected with a prion, e.g. one that causes BSE in cows ('mad cow disease') and CJD in humans. Prion diseases in humans are very rare.

Immunity

An organism develops **immunity** when it is able to defend itself against disease-causing organisms, cancer cells and foreign toxins.

Definition: Immunity is the ability of an organism to defend itself against pathogens, cancer cells and foreign toxins.

Through the ongoing process of **evolution** by **natural selection**, exposure to a variety of pathogens has resulted in the human body having a range of defence mechanisms for protection. The body has different ways of preventing pathogens from entering; however, even if they do gain entry, the human immune system is equipped with many other ways of fighting the disease-causing organisms. This means that infection does not always lead to disease.

Not all disease is caused by infection either. Disease can also be caused by the body's own cells becoming abnormal. **Cancer** can arise when normal body cells undergo uncontrolled cell division forming **tumours** (Chapter 10). Cancer is an example of a **non-infectious disease**.

A variety of cells, mainly **white blood cells**, play a role in our immune system. They work together as part of a defence response that can distinguish between the body's own cells ('self') and the cells of other organisms ('non-self') that might enter the body. White blood cells provide us with a lot of our body's ability to fight disease. Many of these white blood cells can even distinguish between one species or strain of pathogen and another, as well as recognise and respond to infected cells and cancer cells.

Antigens and Antibodies

Pathogens are identified by our immune cells by the presence of **antigens**. Antigens may be **proteins**, **polysaccharides**, **lipids** or **nucleic acids** produced by a pathogen. Antigens are often molecules that are part of the surface of the pathogen (see Fig. 12.1).

In response to the presence of antigens in the body, specialised white blood cells of our immune system produce **antibodies**. A specific antibody protein will bind to a particular antigen due to its complementary shape (see Fig. 12.2) In this way, antibodies disable pathogens and infected cells, preventing them from causing disease.

Definition: Antigens are non-self substances that stimulate the production of specific antibodies and other immune responses.

Definition: Antibodies are proteins produced by specific white blood cells in response to the presence of an antigen.

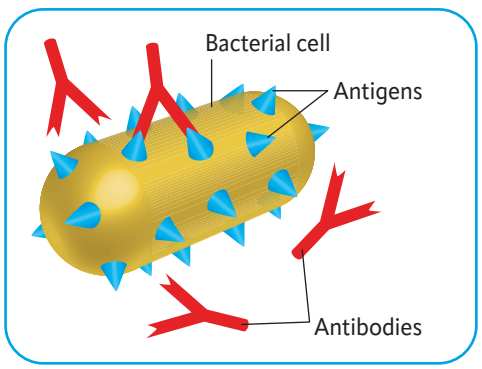


Fig. 12.2 *Antibodies are proteins, produced by specific immune cells, that are complementary in shape to antigens produced by a pathogen. Antibodies bind to the antigen, disabling the pathogen.*

Viruses and Disease

Viruses cause disease by getting inside the cells of the organism they infect (the host), taking over those cells, and disrupting their normal function. As the virus particles spread from cell to cell, and around the body, tissues and organs are negatively affected, resulting in illness. All viruses are therefore referred to as **intracellular** parasites – they invade and infect the cells of the host and use them to produce more virus particles, causing harm in the process.

Viral Replication

As we learned in Chapter 2, viruses are not capable of reproduction as we would use the term for living things. We say that viruses undergo **replication** but even this they cannot do without a host cell. As discussed in the previous section, viruses are **obligate parasites**. They can only function by using the host cell machinery to produce more viral proteins and nucleic acids.

Definition: An **obligate parasite** is an organism (or virus in this case) that must live in or on another organism (the host), causing it harm.

These viral components are then assembled inside the cell, forming many new viral particles. The steps involved in viral replication are outlined below and illustrated in Fig. 12.3.

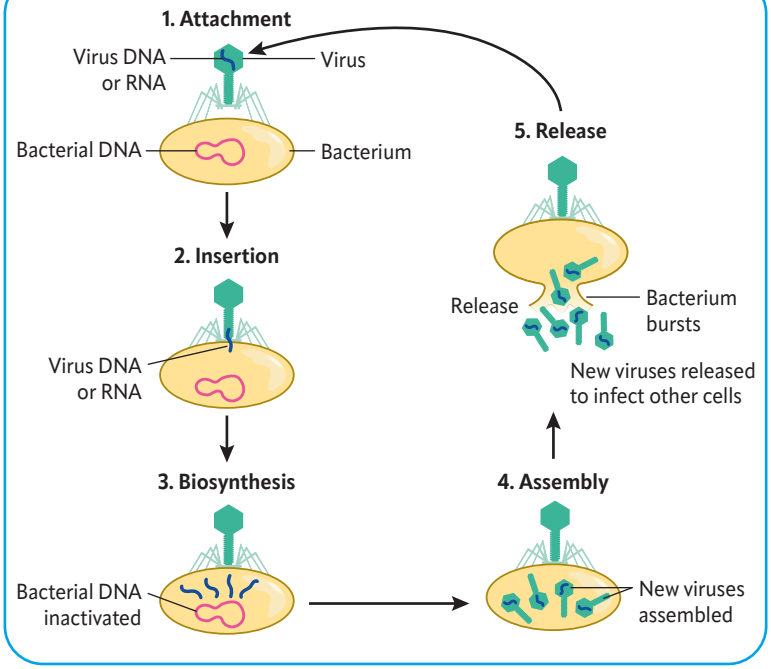


Fig. 12.3 *The stages in the process of viral replication. The diagram shows the infection of a bacterial cell by a bacteriophage (a type of virus that only affects bacteria).*

1 Attachment

To establish an infection, viruses must gain access to the cell interior. They do this by **attaching to the surface of host cells**. Each virus will attach to and infect a specific cell type in the body. For example, SARS-CoV-2, the virus that causes COVID-19, infects cells of the nasal passage and lungs. HIV, the virus that causes AIDS, infects white blood cells, while bacteriophages are viruses that only infect bacterial cells. Virus particles attach to cells by binding to complementary-shaped proteins in the host cell membrane that act as **receptors**.

2 Insertion

Once attached, either the whole virus particle or just the genetic material (either DNA or RNA depending on the virus) is inserted into the cell.

3 Biosynthesis

The viral genetic material is then **replicated** and the genes **expressed** by the host cell. The proteins needed to make new virus particles are synthesised at the host's ribosomes, instead of the proteins normally produced by the host cell.

4 Assembly

New virus particles are assembled. The viral genetic material (DNA or RNA) is enclosed in the **capsid** protein coat.

5 Release

Fully assembled virus particles are then released from the cell. Sometimes the cells bursts, releasing the virus particles. In other cases, as the virus particles leave through the cell membrane, they gain an envelope made from the lipid bilayer.

Influenza and SARS-CoV-2 are examples of viruses that have a lipid envelope. This is why handwashing with soap (detergent) is especially effective against these viruses as it disrupts the lipids.

Link 12.1

Scan this code to see an animation of a bacteriophage replicating.

Mode of Action of DNA and RNA Viruses

Viruses can have either DNA or RNA as their genetic material.

The mode of action of **DNA viruses** involves using the host cell's **DNA polymerase** to transcribe the viral DNA, and replicate in the host cell nucleus. They are typically more stable, have lower mutation rates, and often cause more long-term diseases that are more easily vaccinated against, resulting in longer-term immunity.

RNA viruses differ in their mode of action. They either contain their own **RNA polymerase**, or contain an enzyme called reverse transcriptase that can make a DNA copy from the viral RNA. RNA viruses typically replicate in the cytoplasm. They are usually less stable than DNA viruses, and have a higher mutation rate resulting in a lot of strain variation, which is difficult to vaccinate against. They usually result in short-term infections, but immunity is short-term too.

Table 12.1 *Key differences between DNA and RNA viruses.*

Feature	DNA Viruses	RNA Viruses
Genetic material	Deoxyribonucleic acid (DNA).	Ribonucleic acid (RNA).
Stability	More stable due to the chemical **stability** of DNA.	Less stable because RNA is more **unstable** than DNA.
Mutation rate	**Lower mutation rate** so immunity (via infection or vaccination) is usually longer-lasting.	**Higher mutation rate**, evolve faster, changes mean they are more difficult to vaccinate against.
Disease pattern	Often **longer-term** chronic infections.	Usually acute, **short-term** infections.
Replication location	Usually replicate in the host cell **nucleus**.	Usually replicate in the host cell **cytoplasm**.
Dependence on host	Use host cell's **DNA polymerase** or encode their own.	Use their own **RNA polymerase** or **reverse transcriptase**.
Examples	• Herpesviruses (e.g. HSV). • Poxviruses (e.g. smallpox, monkeypox). • Hepatitis B.	• Coronaviruses (e.g. SARS-CoV-2). • Influenza. • HIV.

The Human Immune System

The human immune system has two different levels of defence: **the innate immune system** and the **adaptive immune system.** The innate immune system provides general defence against infection, while the adaptive immune system consists of specialised white blood cells that work together to defend against specific pathogens. The adaptive immune response involves the production and activity of antibodies produced by specialised white blood cells.

Definition: The **innate immune system** is a non-specific defence system involving a first line of protection against pathogens.

The Innate Immune System

The word 'innate' is used to describe characteristics that we are born with. The innate immune system does not distinguish between different types of pathogens, but is composed of a range of responses that forms the body's first lines of defence against infection. It involves those parts of the body that are exposed to the external environment, such as the skin, the digestive system, the breathing system and the vagina.

Barriers to Entry

Skin

The outermost layer of skin is called the **epidermis** and contains cells that produce keratin. These cells harden and die, forming a protective layer. We also regularly shed these dead cells, and with them any pathogens that may be present on our skin.

Sebaceous glands in the skin (a type of **exocrine** gland) produce **sebum**. Sebum moisturises the skin, which stops it from cracking, preventing pathogen entry. The growth of pathogens on the skin is inhibited by **antimicrobial** substances in the sebum, as well as the **acidity** (low pH) of both sebum and sweat.

The Human Microbiome

The community of microorganisms that colonise our skin, gut, vagina and other surfaces creates a protective barrier against invading pathogens. Many of these **mutualistic** microorganisms produce antimicrobials which helps them to compete with harmful microbes. Members of the human microbiome can also signal immune cells to help defend against pathogens. A balanced microbiome also helps regulate **inflammation** (see also Chapter 20).

Mucous Membranes and Secretions

Pathogens can enter the body at various points that are not covered by skin. Hairs and mucus in the nasal passage help trap pathogens in inhaled air. **Mucus** in the **bronchi** and **trachea** also trap debris such as dust particles and microorganisms. Hair-like **cilia** in these airways move together in a wave-like pattern to carry mucus containing foreign material up and out of the breathing system (Fig. 12.4).

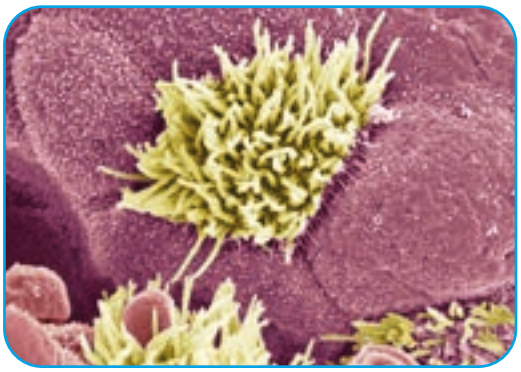


Fig. 12.4 *Coloured scanning electron micrograph (SEM) of the lining of the trachea (windpipe). Its lining contains mucus-secreting cells (pink) and epithelial cells bearing hair-like cilia (white).*

Vaginal mucus provides a protective layer against invading microorganisms. The relatively low pH of the vagina also has an antimicrobial effect. **Lysozyme** is an **antimicrobial** that is found in vaginal mucus, saliva and tears. **Hydrochloric acid** is secreted by the lining of our stomach to kill microorganisms that are present in the food we eat.

Table 12.2 *Mucous membranes and their role in the innate immune system.*

Location of Mucous Membranes	Role
Bronchi and trachea	Traps foreign particles and microbes in inhaled air.
Nasal passages	Traps foreign particles and microbes in inhaled air.
Digestive system	Hydrochloric acid in the stomach mucus kills bacteria.
Vagina	Low pH and lysozyme inhibit the growth of pathogens.
Eyes	Contains lysozyme, an antimicrobial that kills bacteria.

The Inflammatory Response

If a pathogen does breach the body's barriers to infection, it can lead to inflammation. This involves swelling, heat and redness and is often painful. Inflammation is the result of histamine release, increased blood flow to the affected area, and the movement of large numbers of phagocytic white blood cells to the site of injury or infection. The inflammatory response is the body's attempt to contain and eliminate an infection. Inflammation usually subsides once the healing process has begun, as it would otherwise cause too much damage to the body cells.

Clotting

When the skin is broken and blood vessels such as **capillaries** are damaged, **platelets** release clotting factor proteins which form clots and seal off the wound, preventing further entry of pathogens as well as reducing the loss of blood.

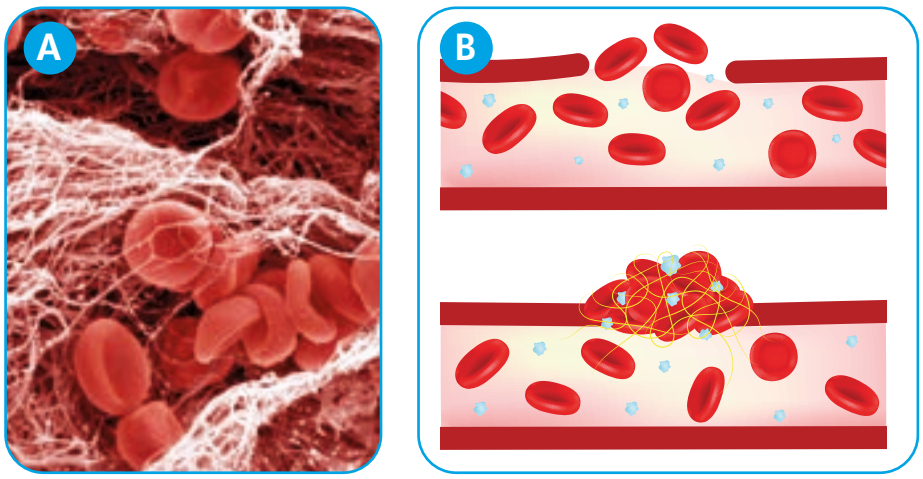


Fig. 12.5 *(A) Coloured scanning electron micrograph (SEM) of a blood clot. Red blood cells are trapped within a protein mesh (cream) formed in response to chemicals secreted by platelets (pink) after (B) injury to a blood vessel. The part below shows a clot forming in response to a break in a blood vessel.*

Histamine

Histamine is a chemical released by damaged tissue cells that causes the capillaries to dilate (widen) and their walls to become more permeable. Increased blood flow through these enlarged capillaries causes the skin to become warm and red. This increase in temperature can inhibit the growth of pathogens and speeds up the production of antibodies by white blood cells.

The increased blood flow also brings more white blood cells to the site of infection. Greater permeability of the capillary wall allows white blood cells and platelets to leave the blood and move into the fluid around the wounded tissue (see Fig. 12.10). This can cause swelling in the affected area (see Fig. 12.6).

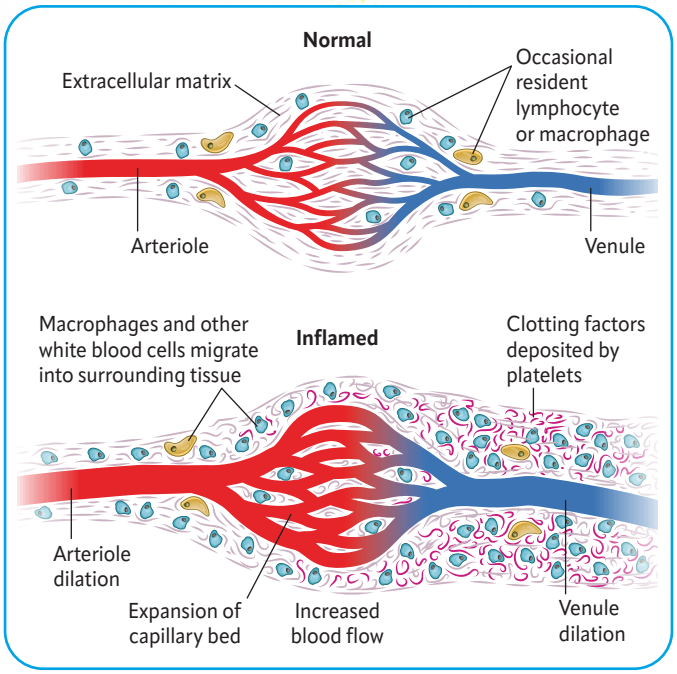


Fig. 12.6 *Increased blood flow leads to inflammation.*

HL

Phagocytic White Blood Cells

There are several different types of white blood cells (Fig. 12.7). Some are phagocytic and are involved in the innate immune response.

Phagocytosis is the process of engulfing microbes, infected cells or **antigens**, destroying them (Figs. 12.8 and 12.9).

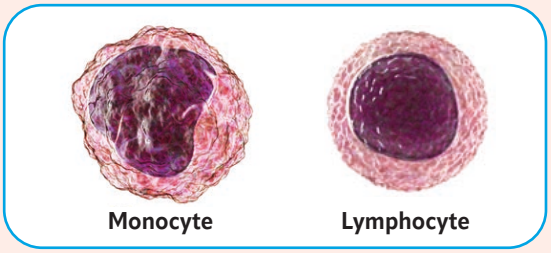


Fig. 12.7 *Different types of white blood cells.*

Phagocytic white blood cells are the first responders – these cells engulf (by phagocytosis) and destroy antigens or foreign cells that they encounter (Figs. 12.8 and 12.9). The yellow-white pus that forms at the site of infection is made up of large number of dead phagocytes. They make up 50–80% of the white blood cells circulating around the body.

Phagocytes also secrete **signalling proteins** that attract other white blood cells to the damaged area.

Monocytes and Macrophages

Monocytes are very large phagocytic white blood cells that circulate in the blood. When monocytes arrive at the site of infection, they are 'recruited' or stimulated by various chemical signals to join in the immune response. They move from the blood into the surrounding tissue at the site of infection where they mature into cells called **macrophages**, which are larger and more aggressive (see Fig. 12.10). Macrophages can also kill body cells that have become infected with a pathogen, by phagocytosis.

Macrophages can enlist the help of other white blood cells such as **lymphocytes** (see page 265) by secreting **signalling proteins** and by **'presenting' antigen** (see Fig. 12.9). This behaviour makes macrophages a crucial **link between the innate and the adaptive immune systems**.

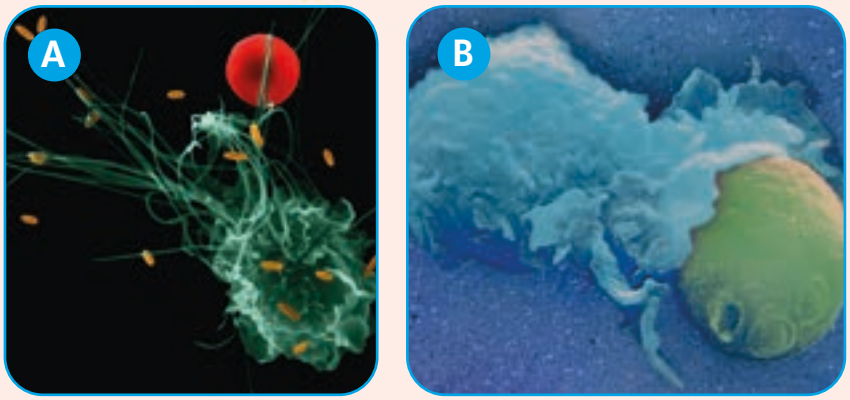


Fig. 12.8 *Scanning electron micrographs (colour-enhanced). (A) A macrophage (green) showing phagocytosis of* E. coli *bacteria (orange). A red blood cell is also shown. Note the macrophage has extensions that aid in finding and trapping bacteria for phagocytosis. (B) A phagocytic macrophage (blue) engulfing a yeast cell (yellow).*

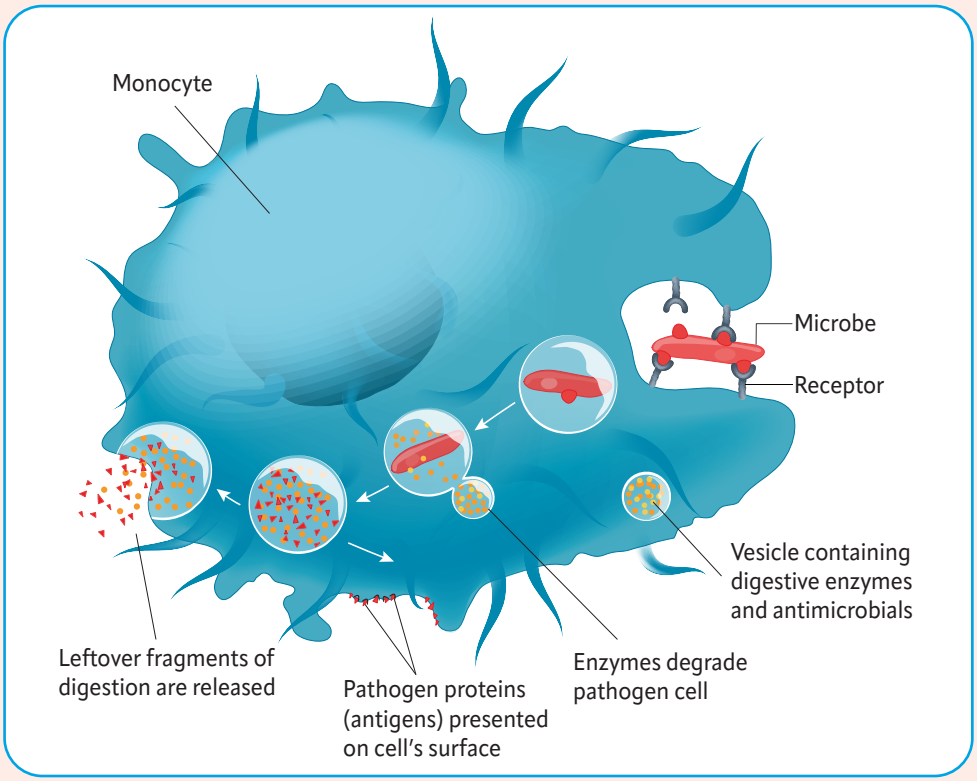
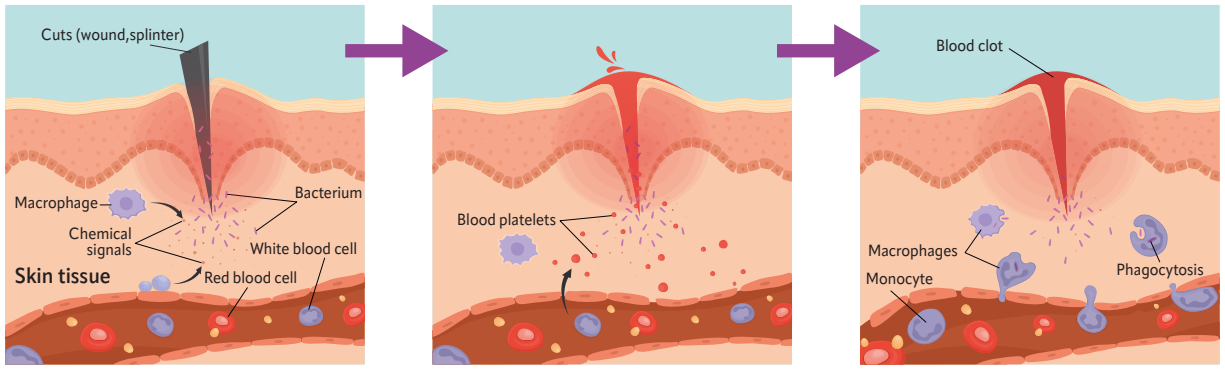


Fig. 12.9 *The process of phagocytosis of a pathogen by a macrophage. An important part of the behaviour of macrophages is their ability to 'present' one or more of the pathogen's antigens on its surface. Antigen-presentation is a crucial behaviour of various white blood cells in the immune response.*



Chemical signals released by activated macrophages at the injury site cause nearby capillaries to widen and become more permeable.

Platelets from blood release blood-clotting proteins at wound site.

Chemical signals released by various types of cells attract monocytes from the blood to the site of the injury.
Macrophages engulf pathogens and the tissue heals.

Fig. 12.10 *The inflammatory response as part of the body's innate immune system. Note that* ***monocytes*** *in the bloodstream are activated and migrate into the tissue to become* ***macrophages****.*

Natural Killer Cells

Natural killer (NK) cells are a type of **lymphocyte**. Most lymphocytes are only involved in the adaptive immune response (see page 265), but NK cells can play a role in both innate and adaptive responses.

NK cells can attach to pathogen-infected cells, **cancer** cells or directly to pathogens. They produce toxins, such as **perforin,** which creates holes in the target cell membrane causing it to rupture and die. NK cells can act immediately, without ever having been exposed to that pathogen before.

NK cells can also work with the adaptive defence system. If a pathogen has been coated with antibody molecules, NK cells can more easily bind to the target cell and kill it with toxins (Fig. 12.11).

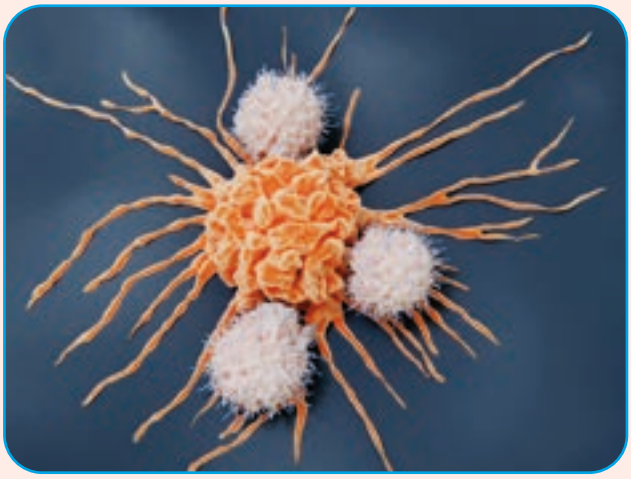


Fig. 12.11 *Natural killer (NK) cells attacking a cancer cell (orange). NK cells target virus-infected cells and tumour cells without having been previously exposed or activated.*

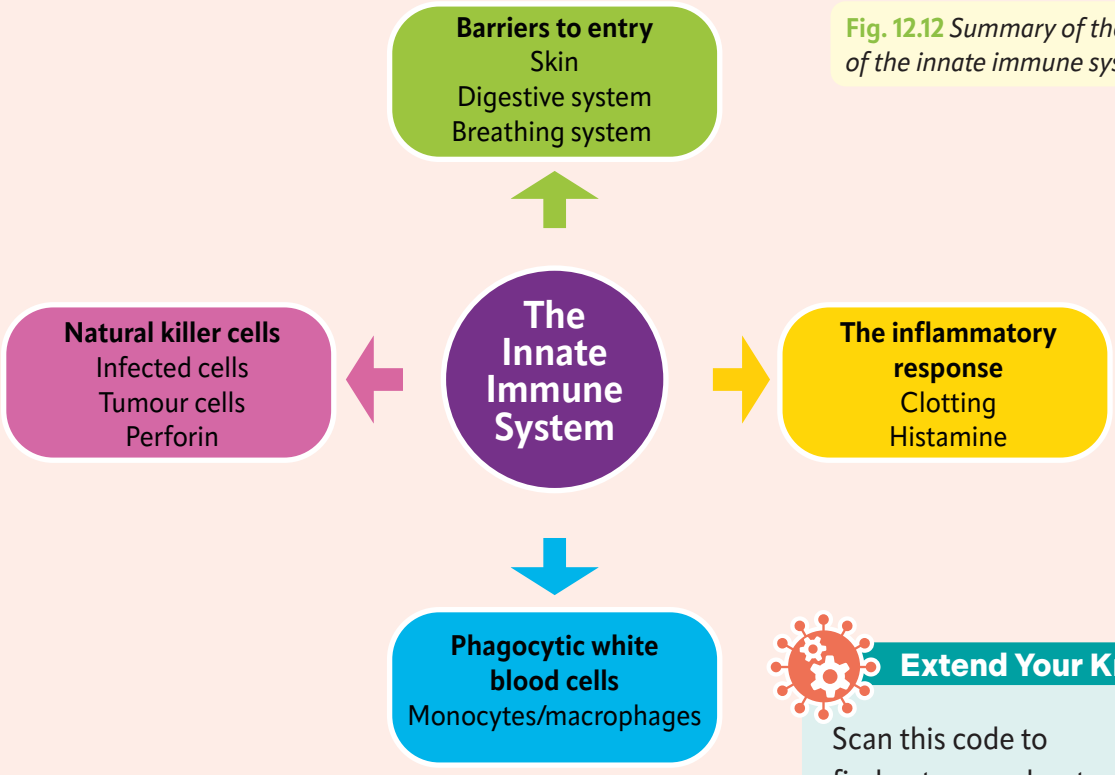


Fig. 12.12 *Summary of the components of the innate immune system.*

Extend Your Knowledge

Scan this code to find out more about how macrophages and NK cells of the innate immune system recognise their targets.

The Adaptive (or Acquired) Immune System

If the non-specific defences of the innate immune system do not prevent infection, the processes of the **adaptive immune system** (also known as the acquired immune system) come into play. This system, only present in vertebrates, can distinguish between different types of antigens and pathogens. The production of **antibodies** by cells of the adaptive immune system is a critical part of the specific nature of the response.

Definition: The **adaptive (or acquired) immune system** is composed of specialised cells and processes that protect against specific pathogens.

It involves a variety of different white blood cells, some of which can customise their response to protect against the nearly limitless variety of pathogens that the body might encounter.

Table 12.3 *Comparison of innate and adaptive immune systems.*

Innate Immune System	Adaptive Immune System
Rapid response.	Slow response.
Occurs in all animals.	Occurs only in vertebrates.
General response against pathogens.	Specific response against pathogens.
Response does not vary upon reinfection.	Response is faster and more efficient upon reinfection.
White blood cells do not produce antibodies.	Some white blood cells produce antibodies.

HL Lymphocytes

Lymphocytes play a key role in the adaptive immune system. They can recognise antigens due to the presence of **receptor proteins** in their cell membranes. Each lymphocyte produces its own specific receptor. It is estimated that there could be as many as 10^{25} different lymphocyte receptors, each one able to bind to specific antigen molecules due to its unique complementary shape. This astounding amount of diversity enables lymphocytes to recognise and distinguish between the millions of different possible antigens that we may encounter during our lifetime.

Two classes of lymphocyte are primarily involved in the adaptive immune response: **B lymphocytes** and **T lymphocytes**. B lymphocytes are more involved in fighting free pathogens, like bacteria and viruses, while T lymphocytes mainly target body cells that are infected or abnormal. The lymphatic system is a network of vessels containing **lymph**, a clear fluid that transports white blood cells throughout the body.

B Lymphocytes

B lymphocytes (B cells) are produced in the **bone marrow** and mature there before migrating to the blood and the lymph. B cells can produce antibodies but only do so when **activated**. If a specific antigen binds to the receptor protein (called a **B cell receptor**) on the membrane of a circulating B cell, this activates the B cell. The **activated B cell** quickly divides many times to form a population in which each new cell is a clone of the original activated B cell. Many of these B cells become **plasma cells**, while others become **memory cells** (see Fig. 12.13).

Plasma Cells

Plasma cells can **secrete** their B cell receptor proteins so that they separate from the cell membrane. They are then known as **antibody** molecules. Antibodies are capable of binding to specific antigens due to their complementary shapes.

The antibody produced by a plasma cell is identical to the B cell receptor protein, but instead of remaining attached to the surface of the B cell it is released and circulates freely around the body. Each plasma cell can produce and secrete large numbers of antibody molecules.

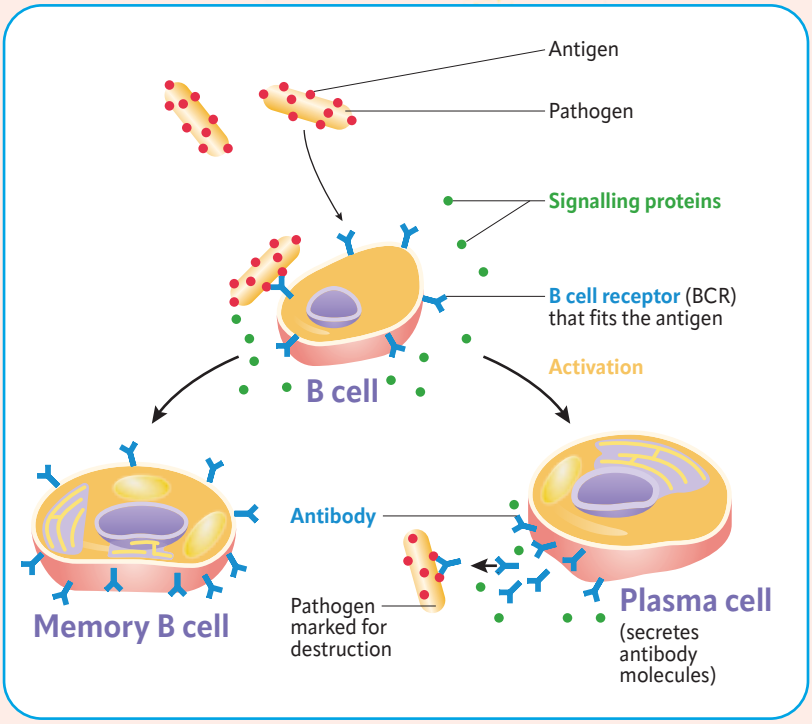


Fig. 12.13 *B cell receptors. Each B cell has a unique receptor protein. When a B cell encounters an antigen that binds to the B cell receptor due to its complementary shape, it becomes activated and differentiates into either a plasma cell or a memory B cell.*

Signalling proteins are also involved in activating B cells and stimulating them to multiply. Some of these signalling proteins are produced by cells in the **innate immune system** (e.g. monocytes and macrophages). Once activated, B cells also produce signalling proteins that stimulate other B cells. B cells can also produce multiple signalling proteins that are implicated in **autoimmune diseases** (see page 281).

The Role of Antibodies

Antibody molecules produced by a plasma cell bind to antigens that are on the surface of the pathogen itself. These bound antibodies interfere with a pathogen's ability to infect a host cell in various ways (see Fig. 12.14).

1. Coated pathogen cells are unable to interact with host cells. In this way, the bound antibody **neutralises the pathogen**, preventing further infection.

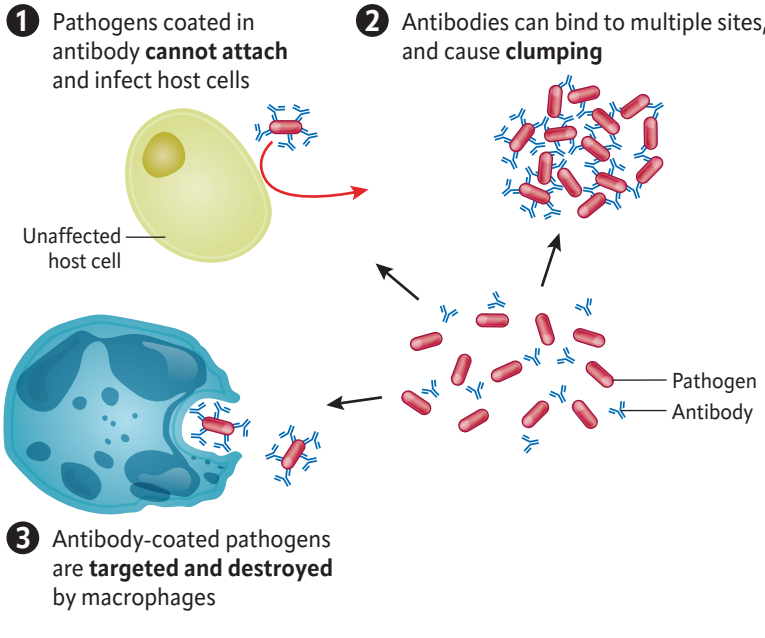


Fig. 12.14 *Antibodies have many different roles in helping to defeat pathogens.*

2. Antibodies can bind more than one antigen molecule at the same time, which can cause viruses or pathogen cells to **clump** together. Clumped viruses and cells cannot infect host cells and are more readily engulfed by phagocytes.
3. Pathogens that are coated in antibody are more readily engulfed by **macrophages** at the site of infection.

Memory B Cells

It usually takes about 4–7 days after exposure to antigen before antibody levels are detectable. It can take 1–2 weeks before this **primary immune response** is fully developed with high levels of antibodies and activated lymphocytes. Having a large population of antibody-producing plasma cells is central to the success of the adaptive immune system in dealing with an infection.

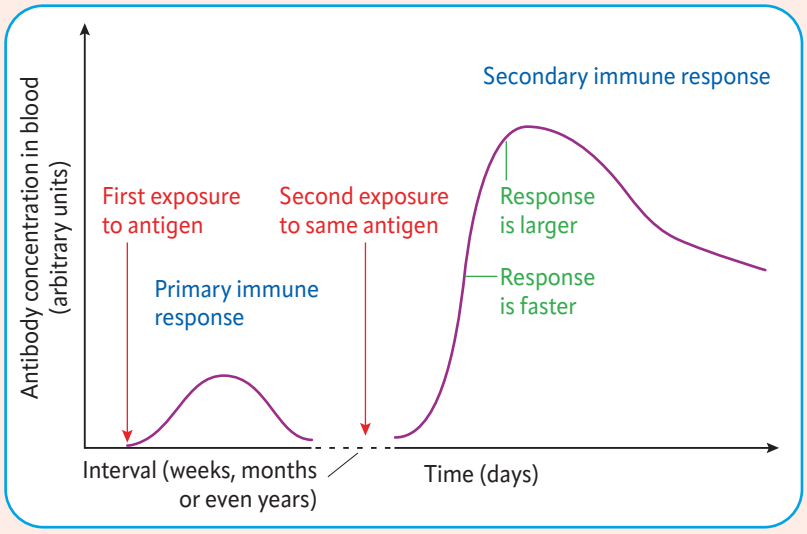


Fig. 12.15 *Antibody production in primary and secondary responses. The secondary response could be soon after the primary response or it may be months or years later.*

Once the infection has been overcome, the population of plasma cells dies off, leaving behind a small number of **memory B cells**. These cells allow the body to recognise the antigen if it is encountered again in the future.

This is known as a **secondary immune response**, and it is faster and more efficient than the primary immune response (see Fig. 12.15). Memory B cells rapidly divide and proliferate to produce a large population of antibody-producing cells. The presence of memory B cells (and memory T cells; see T lymphocytes section page 268) in the body gives an individual an **immunological memory**.

Link 12.2

Scan this code to see an animation of antibodies binding to the antigens on a virus.

T Lymphocytes

T lymphocytes (T cells) mature in the thymus before migrating in the blood and the lymph. T cells do not produce antibodies, but they do play a central role in the immune response. Some T cells regulate the immune response, while others directly attack cells that are presenting antigens.

Antigen-Presentation

T cells are unable to recognise antigens in the same way as B cells do. Instead, T cells require an antigen to be 'presented' to them by other cells such as a **macrophage** (see Monocytes and Macrophages page 262).

When a macrophage engulfs and digests a pathogen, the antigen is sent to the surface of the macrophage where it is displayed. This behaviour is called **antigen-presentation** (see Fig. 12.9). Activated B cells can also present antigen to T cells.

When a T cell encounters an antigen-presenting cell, it becomes activated, dividing repeatedly to produce clones of itself, all of which respond rapidly to the antigen that has entered the body (see Fig. 12.17).

In this way, information about the pathogen is passed by the antigen-presenting cells of the innate immune system to the lymphocytes of the adaptive immune system, **linking the two immune systems**.

Cancer cells and virus-infected cells can also present abnormal proteins on their surface, so T lymphocytes can also recognise these cells as being a problem, even though they are 'self'.

Types of T Lymphocytes

- **Killer T cells** kill cancer cells, cells that are infected with a pathogen, or cells that are otherwise damaged. By recognising antigens on the surface of these abnormal cells, killer T cells bind to the virus-infected cell or cancer cell. Killer T cells produce **perforin**, which forms holes in the target cell's plasma membrane causing the abnormal cell to die.
- **Helper T cells** help to regulate the immune response by secreting **signalling proteins** that stimulate B cells and killer T cells. Some types of helper T cells also bind to antigen-presenting phagocytic cells and enhance the cell's activity. Activated helper T cells stimulate B cells to become activated, multiply and produce antibodies (see Fig. 12.17). These activated helper T cells can also secrete signalling proteins to recruit more **macrophages** to the site of infection, thus increasing the inflammatory response (see page 261).
- **Suppressor T cells** modulate the immune system, ensuring that the body does not respond inappropriately. Suppressor T cells have a role in dampening the immune response once an infection is under control. They also play a part in preventing an overreaction by the immune system to various environmental antigens (such as pollen, dust and gut microbes). In doing so, suppressor T cells maintain the immune system's ability to distinguish between self and non-self proteins. They also help avoid the costly production of excess antibody.
- **Memory T cells**, in a similar way to memory B cells, can live for many years and can launch a quick immune response to an antigen that the body has had to deal with in the past.

Table 12.4 *Summary of T cell roles.*

Type of T Cell	Role	Key Features
Helper T cells	Coordinate the immune response by activating other immune cells. Stimulate B cells to produce antibodies.	Release signalling proteins to stimulate B cells, killer T cells and macrophages. Activated by antigen-presenting cells.
Killer T cells	Destroy infected, cancerous or foreign cells directly. Release perforin to kill target cells.	Activated by antigen-presenting cells. Essential for controlling viral infections and tumour growth.
Suppressor T cells	Inhibit activity of other immune cells to avoid excessive inflammation. Prevent inappropriate **autoimmune** response by other immune cells.	Can release signalling proteins that inhibit the activity of other T cells and B cells. Regulate immune tolerance to foreign antigens such as food proteins.
Memory T cells	Provide long-lasting immunity to past infections. Can be either helper or killer T cells in function.	Quick response on re-exposure to the same pathogen. Persist for years or even a lifetime after an infection or vaccination.

Natural Killer Cells

As we have seen, natural killer (NK) cells are lymphocytes that play an important role in innate immunity (see page 260) by destroying **virus-infected** cells and **tumour** cells. However, recent evidence suggests that some NK cells can also behave more like lymphocytes of the adaptive immune system. NK cells have been shown to respond more effectively when they encounter an antigen for the second time. These cells have become known as 'memory NK cells', as they seem to be able to recognise specific antigens, unlike regular NK cells.

NK cells produce signalling proteins that influence T cells and B cells. In this way, NK cells serve as a **bridge** between the innate and the adaptive immune systems.

Lymphocyte Interactions

There are many ways in which lymphocytes interact with one another, and with other immune cells, to enhance and accelerate the immune response.

- T cells encountering antigen-presenting macrophages become activated and multiply.
- B cells also present antigen to helper T cells.
- Helper T cells bind to B cell receptors and this binding stimulates the T cell.
- Activated helper T cells respond by releasing signalling proteins, which fully activate the B cells and killer T cells.

The roles and interactions of macrophages (former monocytes) and lymphocytes are summarised in Figs. 12.16 and 12.17.

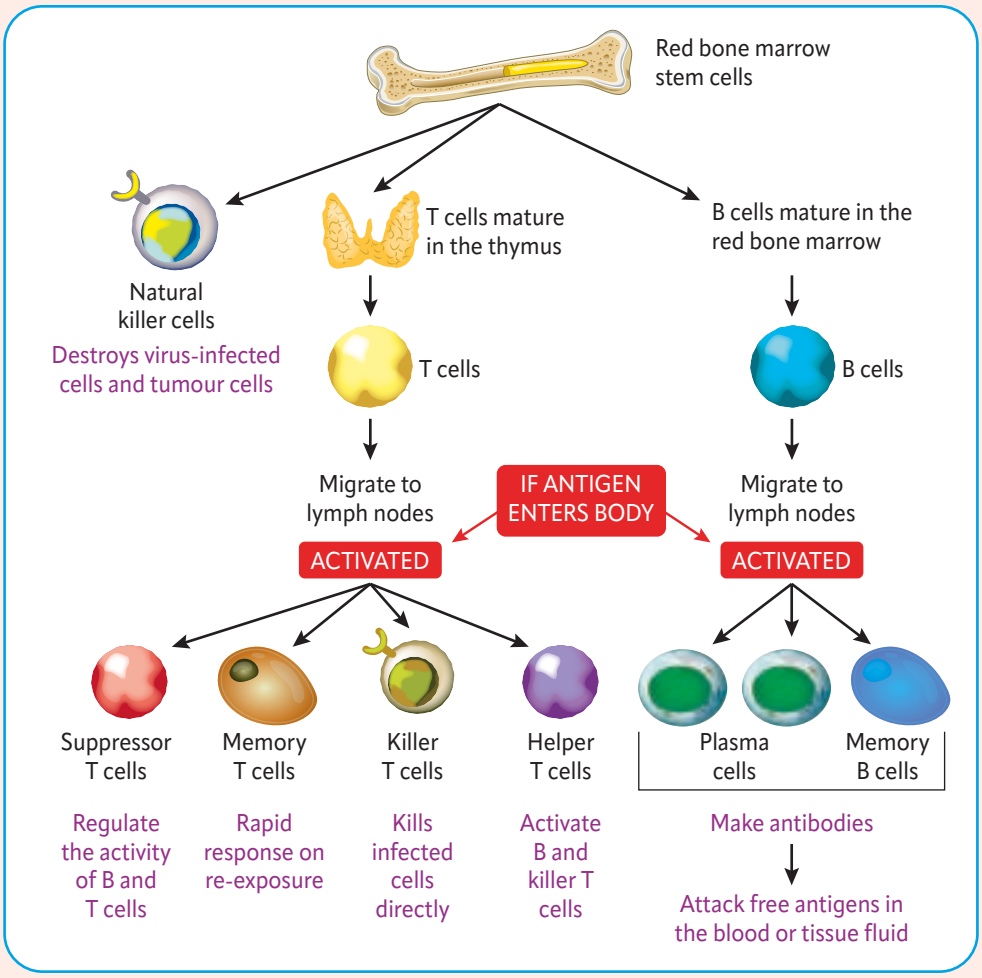


Fig. 12.16 *Summary of the production, maturation and roles of lymphocytes.*

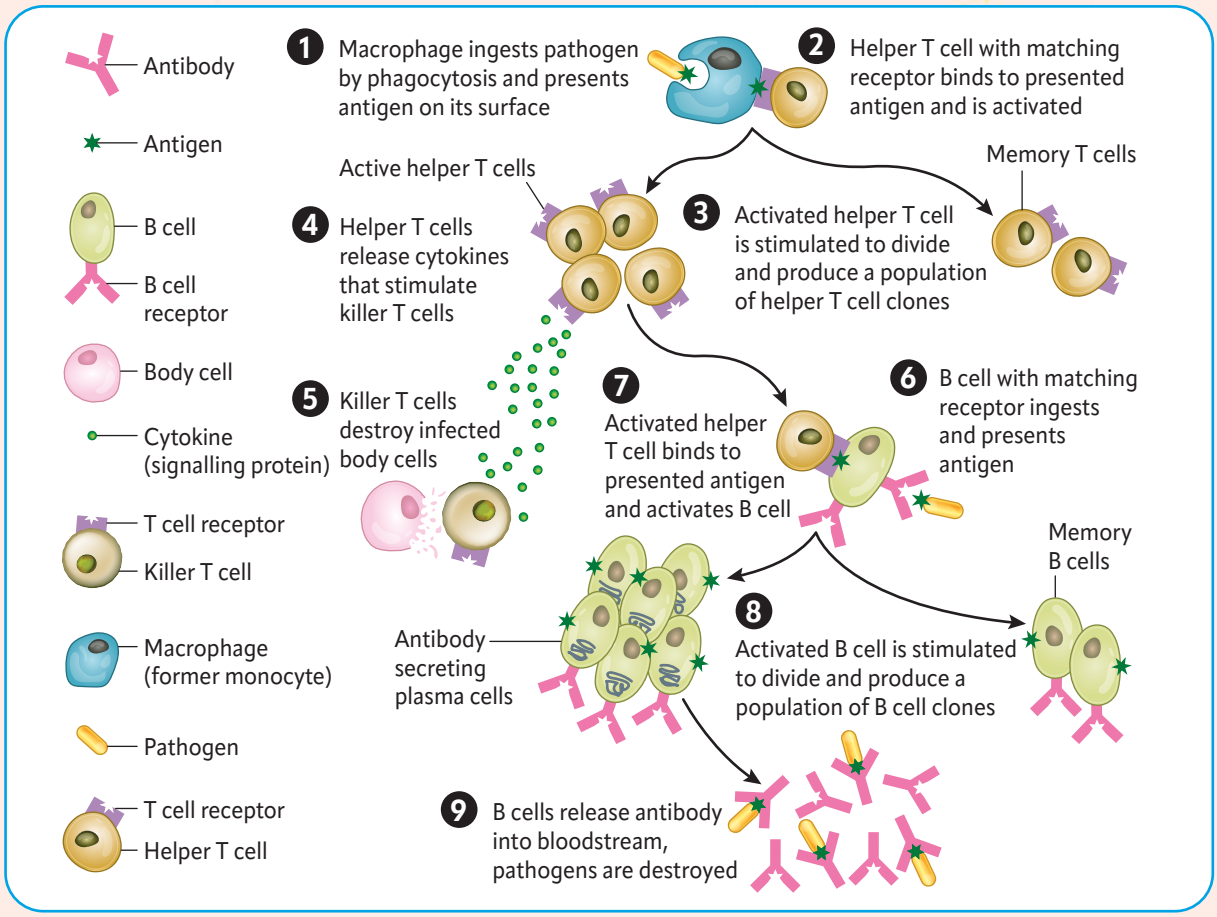


Fig. 12.17 *Summary of the interaction between macrophages (former monocytes) and lymphocytes in the adaptive immune system.*

Active and Passive Immunity

As we have seen, the adaptive immune response following infection usually provides long-lasting protection against a specific **pathogen**. While immunity acquired against some pathogens can last a lifetime (e.g. measles virus), immunity to others (e.g. SARS-CoV-2) may not last quite as long. Immunity can also be acquired by the body through passively receiving antibodies from a source outside itself. This kind of acquired immunity is not long-lasting.

Definition: Active immunity is acquired when our body's own immune cells produce antibodies in response to the presence of an antigen.

Active Acquired Immunity

Active immunity involves the body producing its own antibodies. Lymphocytes are activated by antigens on the surface of pathogens, and antibodies are produced by the plasma cells. It takes some time for enough B and T lymphocytes to be produced to mount an effective response to a first-time infection. Memory cells survive and allow a quicker and more efficient response if the same pathogen is encountered again.

Active immunity can be acquired **naturally** or **artificially**.

Natural Active Immunity

Natural active immunity is acquired when a person gets an **infection** and the immune system responds, activating lymphocytes, producing antibodies and defeating the pathogen. The person experiences the normal symptoms of the disease and recovers, for example when someone gets chickenpox or flu.

Artificial Active Immunity

Artificial active immunity is acquired when a person is given a dose of inactivated pathogen (or a part of the pathogen) and the immune system responds by activating T and B lymphocytes and producing antibodies. This is also known as **vaccination**.

Definition: Vaccination involves introducing a non-harmful inactivated pathogen (or antigen from a pathogen) that provides active acquired immunity.

Passive Acquired Immunity

With passive immunity, antibodies appear immediately in the blood but protection is often only temporary, lasting weeks. Passive immunity can be acquired naturally or it can be acquired artificially.

Definition: Passive immunity is acquired when a person receives antibodies made by another organism.

Natural passive immunity is acquired when **maternal** antibodies are transferred to the foetus through the **placenta**, or to a baby in breast milk (especially **colostrum**) which is rich in antibodies.

Artificial passive immunity is acquired when antibodies specific to a pathogen or toxin are obtained from the blood of an immune animal or person. The antibodies are usually injected, e.g. anti-tetanus injection. Antibodies appear immediately in the blood, so this method of **immunisation** (see next section) provides fast but short-term protection as the antibodies are broken down in the body.

Table 12.5 *Comparison of active and passive immunity.*

	Active Immunity	Passive Immunity
Definition	Acquired by the body making its own antibodies.	Acquired through the transfer of antibodies from another source.
Source of antibodies	Antibodies produced by the body's plasma cells in response to the presence of an antigen.	Antibodies obtained from another person or animal.
Onset of protection	Slow (immune cells require time to produce sufficient antibodies).	Fast (antibodies are immediately available in the body).
Duration of protection	Long-lasting (sometimes lifelong).	Short-term (weeks to months).
Examples	Vaccination, infection.	Breastfeeding, antibody therapy.
Memory	Memory T cells and memory B cells persist.	No immune memory developed.

Prevention of Infectious Diseases

Immunisation

How Vaccines Work

Vaccination involves administering to a person a non-harmful weakened or inactivated dose of a pathogen, or an antigen from the pathogen. This does not cause disease, but instead induces an active immune response. Lymphocytes are activated, antibodies are produced, and memory cells remain in the body for some time after the vaccination. Active immunity resulting from vaccination is usually long-lasting, although a second dose of vaccine (a **'booster'**) is sometimes required to provide long-term immunity. Fig. 12.15 (page 267) shows why this booster is effective – the antibody concentration is much higher after a booster than after the first exposure to the vaccine.

Definition: **Immunisation** is the process in which a person is made immune to a disease.

Definition: A **vaccine** is a biological preparation that provides active acquired immunity to disease.

Go to page 137 in the **Research and Investigations Book** for an activity on Edward Jenner's discovery of vaccination.

Vaccine Safety

Vaccines are given to millions of healthy people worldwide every year, so very high safety standards must be met before they can be used. Vaccines are extensively tested before they are recommended for use. This process can take several years and includes clinical trials with up to thousands of volunteers. Only when a vaccine is shown to be safe and effective is it granted a license.

Some individuals can experience mild side effects after receiving a vaccine. Serious side effects can occur, as with all medicines, but are extremely rare.

Public Health Measures

Sanitation and Hygiene

Sanitation involves public services to keep places clean by removing and treating wastewater, collecting refuse, cleaning the streets, etc. Diseases can spread more readily if sanitation is not carried out properly.

Fig. 12.18 *Refuse collection is an important public health measure.*

Hygiene means keeping yourself and your surroundings clean to avoid getting ill or spreading disease. **Handwashing** with soap is a key part of this. A major public health concern is that infectious diseases affect children more frequently as they may have less awareness of how diseases spread. **Hand sanitising** with gels containing alcohol became commonplace during the COVID-19 pandemic, supplementing handwashing, although handwashing with soap is more effective.

Fig. 12.19 *Handwashing using soap and hand sanitising gel are important hygiene measures.*

Go to page 129 in the **Research and Investigations Book** for a modelling activity on handwashing limiting the spread of infectious diseases.

Proper **food handling** includes cooking food at the correct temperature for long enough, cleaning utensils and surfaces, safe food storage, and avoiding cross-contamination of other food with raw meat. Public health regulations are applied to restaurants and food outlets regarding food preparation, storage and transport to prevent the spread of food-borne diseases caused by bacteria and viruses.

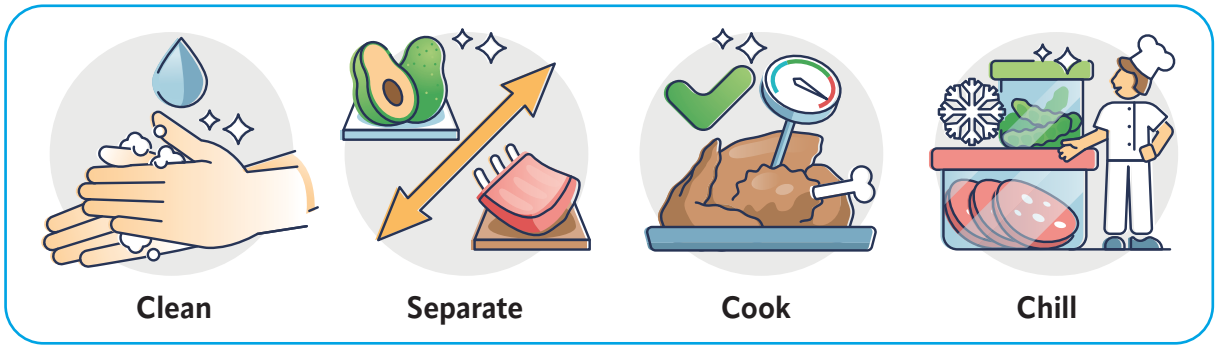


Fig. 12.20 *Four steps for food safety.*

Vector Control

In many areas around the world, vector control is important. **Vectors** are organisms that can transmit diseases. Mosquitos, fleas and rodents are examples of vectors that can carry a number of different microbial infections. Proper disposal of refuse, wastewater management and domestic hygiene are all measures that help reduce the ability of vectors to transmit disease. Insecticides and rodent poisons may also be used to control vector numbers.

Prion Disease Control

Prion diseases (e.g. CJD, see page 257) in humans are very rare, but there is no cure. Symptoms usually progress rapidly and are always fatal. Since they are spread to humans by eating meat from an infected animal, prion diseases in livestock are carefully monitored and controlled. This involves routine screening, the banning of feeding animal-derived protein to livestock, and the culling of infected or at-risk animals.

Education

Fig. 12.21 *Mask-wearing and social-distancing were mandatory indoors in many countries during the COVID-19 pandemic.*

Effective education about how hygiene prevents the spread of infectious diseases plays a crucial role in prevention. The risk of water- and food-borne illness is reduced if individuals are trained in proper hygienic practices in the home and in workplaces. Education also empowers people to take control of their own health and make informed decisions to protect themselves and their communities. Various large-scale research studies around the world have demonstrated that **mask-wearing** reduces the spread of infectious disease. During the COVID-19 pandemic, mask-wearing indoors in public places was mandatory in many countries. Despite misinformation on social media, the majority of citizens were well-informed about measures to prevent the risk of disease to themselves, their families and their communities. The effectiveness and importance of vaccination, mask-wearing, hygiene and **social distancing** were well understood by the general public as they were supported by evidence.

Surveillance

Public health organisations such as the World Health Organisation (WHO) carry out **surveillance**. This involves investigating outbreaks by collecting and analysing data when there is a sudden increase in cases of a particular disease. Surveillance includes real-time tracking of reported cases, genomic sequencing to monitor variants arising from mutations, and wastewater testing to detect spread within the community.

By monitoring trends and patterns in the incidences of infectious diseases, organisations like the WHO can develop models to predict how they spread. This can also inform public health policy, vaccination strategies, and government spending. Surveillance is especially important when rapid decision-making is required, as we saw during the global COVID-19 pandemic. It helps identify the source of the disease and take steps to prevent its spread then and in the future.

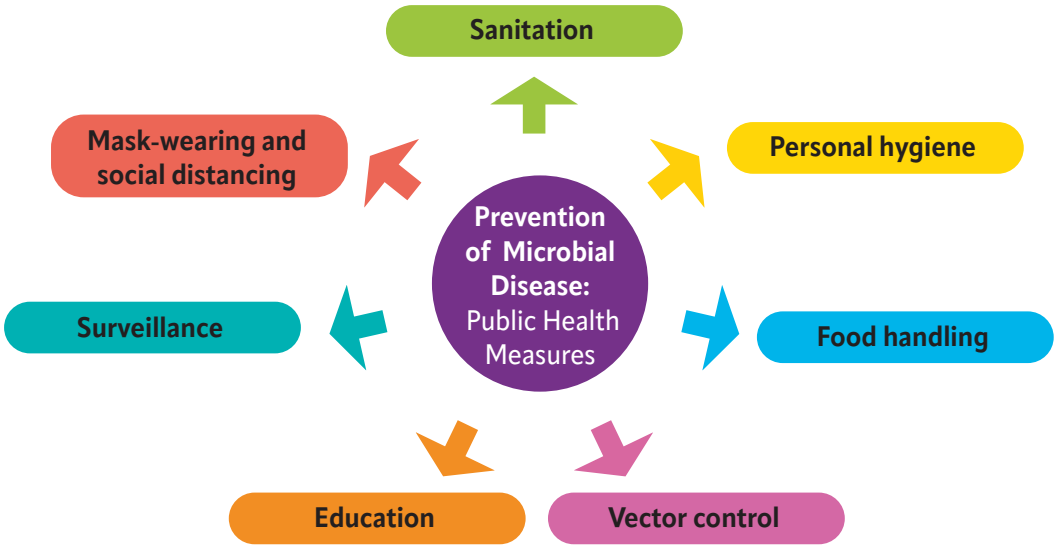


Fig. 12.22 *Prevention of microbial disease – public health measures.*

Treatment of Infectious Diseases – Antimicrobial Medicines

Antibiotics

Antibiotics are chemicals that are effective against bacteria and used in medicine to treat bacterial infections. Fungi and bacteria often naturally produce antibiotics that have no effect on themselves but kill or inhibit the bacteria around them. It is a strategy that microorganisms use to compete with other microorganisms. While antibiotics occur naturally, many of the antibiotics used today are modified or synthetic versions of antibiotics originally isolated from bacteria and fungi.

The first antibiotic to be discovered was **penicillin**. Since then, antibiotics have become one of the most important medicines in human healthcare. Until they were discovered, many common diseases resulted in the deaths of large numbers of people. Penicillin was not available in World War 1 but is estimated to have saved at least 300,000 allied soldiers lives in World War 2. Without antibiotics, soldiers often died from wound infections, sepsis and pneumonia. Antibiotics have since saved countless lives.

Definition: Antibiotics are chemicals produced by microorganisms that kill or inhibit the growth of bacteria.

Fig. 12.23 *Mass production of the antibiotic penicillin began in 1943, in time to save hundreds of thousands of lives during WW2 alone.*

Antivirals

Antibiotics are used to treat bacterial infections, but they are ineffective against viruses. Viruses are not composed of cells and do not have their own metabolism. **Antiviral** medicines are available to treat some viral infections and can work by either interfering with the ability of the virus particles to infect cells, or to replicate, or by boosting the patient's immune system. Effective and safe antiviral compounds are hard to find because they can be harmful to the host cell containing the virus too. A combination of antiviral medications developed to treat HIV has allowed people with the virus to live long and healthy lives.

Fig. 12.24 *Acyclovir is an example of an antiviral medicine.*

Extend Your Knowledge

Scan this code to find out how antibiotics work and why they do not work on resistant bacteria.

Extend Your Knowledge

Scan this code to learn about Alexander Fleming's discovery of penicillin.

Acyclovir is an example of an antiviral used in the treatment of several viral diseases. It is the active ingredient in cold sore creams as it is effective against the *Herpes simplex* virus. It works by inactivating the *Herpes* virus **DNA polymerase**, so the virus cannot replicate.

Antifungals

Fungi can cause skin conditions such as athlete's foot, ringworm and yeast infections such as thrush. These conditions are common but are rarely serious or life-threatening. People with weakened immune systems can suffer more serious illnesses due to fungal infection. **Antifungals** are generally applied as powders or creams, but also as suppositories and tablets.

Fig. 12.25 *Athlete's foot can be treated with an antifungal.*

Antiparasitics

Antiparasitics are a class of medications used to treat diseases such as those caused by various types of parasites (see page 256). **Parasites** may live either inside or on the outside of the host organism. As this is a diverse group of organisms, there are different medications for each type of parasite. Overuse or misuse of these can lead to the development of parasites that are resistant to these treatments.

The Emergence and Spread of Infectious Diseases

Since the 1970s, about 40 infectious diseases have emerged, including AIDS, SARS, MERS, Ebola, avian flu, swine flu, Zika and, most recently, COVID-19. In 2007, the World Health Organisation (WHO) warned that infectious diseases are emerging at a rate that has not been seen before.

Definition: An **emerging infectious disease** is one that has only recently appeared in a population or is rapidly increasing in certain parts of the world.

Factors Affecting the Emergence and Spread of Infectious Diseases

Persistence of the Pathogen

Many emerging and re-emerging diseases arise when infectious agents in **animals** are passed to humans. Some animals can be natural hosts for viruses that cause human disease, acting as **reservoirs** that can persist for a long time. When humans encounter these new **pathogens**, their immune system does not recognise them, and no one has memory cells to fight these infections, so they spread widely. There are also no vaccines or antivirals for these new diseases.

Animal host reservoirs can also provide viruses with the time to mutate into new strains. In Ireland, badgers are vaccinated against bovine TB. This has been shown to be effective at preventing the inadvertent spread of the disease from badgers to cattle. **Vaccination** has recently been shown to be at least as effective as culling badgers, which are a protected species. 5000 badgers were vaccinated in the first half of 2024 and it is hoped that this will reduce the numbers culled.

Long-term infections in people can also provide the virus with increased opportunity to mutate so that new variants arise that are more transmissible or not well-recognised by people's immune systems. In some cases, people can become infected with a virus but do not have any symptoms of the disease. Despite being asymptomatic, they may still transmit the virus to others.

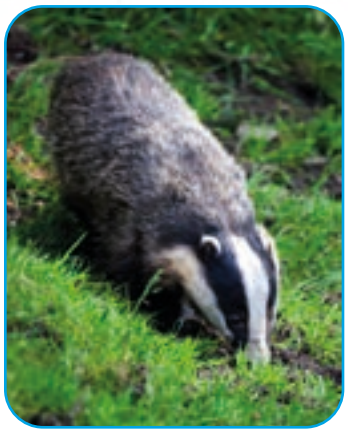


Fig. 12.26 *Vaccinating badgers with the BCG vaccine is effective in reducing the spread of TB to cattle.*

Pathogen Mutation and Adaptation

Viruses have a high mutation rate. Mutations are common during replication of viral DNA, and viral replication happens every time a virus infects a cell. These **mutations** can lead to the emergence of new strains that are more transmissible, or able to evade the immune system.

SARS-CoV-2 and influenza are examples of viruses that have a high mutation rate. As a result, new strains of the influenza virus occur annually around the world, some of which have caused serious illness. New versions of vaccines are required every year to maintain protection of the population from these kinds of rapidly mutating viruses.

Movement of Organisms

Easier, faster travel has allowed infections to spread more rapidly and over greater distances.

Local Movement

An increasing global population has coincided with more densely populated urban centres, and more crowded public transport. The presence and sale of domestic and wild animals in local food markets can also spread disease (see Fig. 12.27).

Regional Movement

Daily commutes within a region can bring infected individuals into contact with new populations, potentially seeding outbreaks in new areas. People involved in farming or hunting can unintentionally introduce plant and animal pathogens from rural areas into nearby towns and cities.

Outbreaks of diseases such as HIV/AIDS and Ebola are thought to originate from infected **bushmeat** (meat from wild animals including primates, birds and reptiles). Bushmeat is a source of sustenance in rural areas and is considered to be a delicacy in bigger towns and cities of West and Central Africa, and parts of South America and Asia. The trade in bushmeat is banned in many countries, and often involves endangered or protected species.

Fig. 12.27 *Turtles in a black market in Asia waiting to be sold for meat.*

Global Movement

Increased **air travel** allows infected individuals to travel vast distances in short periods, potentially carrying diseases to new continents and starting outbreaks. The outbreak of SARS, caused by a coronavirus, in 2003 originated in a colony of horseshoe bats in a cave in rural southern China, but quickly spread to Canada, the USA, Singapore, Mongolia, Taiwan and Hong Kong.

The **global trade** of animals and animal products can introduce new pathogens to regions where they were previously absent. This can be the case with livestock, exotic pets or animal products such as processed meats. Restrictions and regulations regarding the trade and transport of plants and animals are an important part of controlling the emergence and spread of infectious diseases.

As average global temperatures rise due to **climate change**, the geographic range of some disease-carrying insects, like mosquitoes, may expand, allowing them to transmit diseases to new areas.

Global trade of plants and plant products has caused the spread of non-native **plant pathogens**. These are often fungi or viruses that can infect native plants that are not adapted to defend themselves against these new infections. This can result in the spread of disease among native plant species, as has happened in Ireland and Europe in the case of **ash dieback** caused by a fungal pathogen (see Fig. 12.28).

Dutch elm disease is a devastating fungal disease which is spread by elm bark beetles. First detected in Ireland in the 1970s, it is thought to have arrived through the importation of infected timber. It has severely reduced the native wych elm population, leading to the decline of mature elm trees across the country. It remains a major threat to Ireland's elm woodlands.

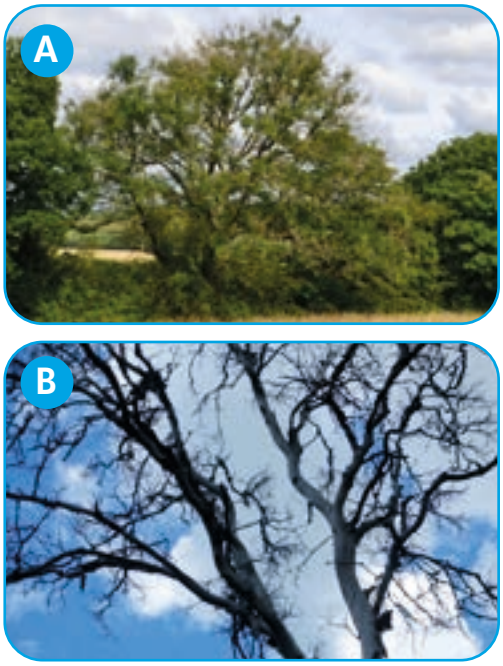


Fig. 12.28 *(A) Dying ash tree that was infected by ash dieback fungus. (B) Dead native Irish wych elm tree infected with the fungus that causes Dutch elm disease.*

Go to page 133 in the **Research and Investigations Book** for an activity on the ongoing problem of ash tree dieback disease in Ireland.

Antibiotic and Chemical Resistance

In recent years, **antibiotic-resistant** bacteria have become a worldwide problem. The widespread use and overuse of antibiotics have resulted in the evolution of strains of bacteria that are able to survive many of the antibiotics that we have relied on to treat routine bacterial infections.

Definition: Antibiotic resistance is the ability of a bacterium to survive the action of an antibiotic.

Over-prescription of antibiotics by doctors, patients not completing courses of antibiotics, and the use of antibiotics in animal feeds to increase the weight of livestock are all factors that have contributed to the spread of antibiotic resistance.

As we saw in Chapter 6, bacteria with mutations for antibiotic resistance are being **selected** by the widespread presence of antibiotics, resulting in bacteria that now have **adaptations** to survive antibiotic treatment. A similar mechanism results in insect and fungal resistance to chemical pesticides with the widespread and continued use of these in agriculture.

Fig. 12.29 Plasmodium vivax*, one of the species of protozoan (a type of protist) that causes malaria, is transmitted to people through the bites of infected female Anopheles mosquitos (pictured).*

Antibiotic resistance is also leading to the emergence of bacteria that are resistant to multiple antibiotics, such as methicillin-resistant *Staphylococcus aureus* **(MRSA)**. Some diseases such as tuberculosis (TB) are re-emerging due to antibiotic-resistant strains of *Mycobacterium tuberculosis*, and a decline in vaccination rates, particularly in countries where TB became uncommon. Medical research is ongoing to find new kinds of antibiotics.

A similar situation can arise with the misuse or overuse of insecticides used to control the insect **vectors** that transmit diseases. Malaria (caused by a microscopic protist), dengue fever, chikungunya and Zika (all caused by viruses) are transmitted primarily by biting mosquitos carrying the pathogen.

Immunity Level Present in the Population

Vaccinated people are much less likely to spread the pathogen to others. If enough of the population is vaccinated it is more difficult for a disease to spread from one person to the next, as most people have some immunity. This is known as **'herd immunity'**. The fact that the disease cannot spread means that vulnerable members of the population, such as newborn babies or those with a weak immune system, are protected from infection (Fig. 12.30).

Measles, for example, is a serious illness. It is very contagious and requires at least a 95% uptake of the vaccine within a population for effective herd immunity. Before widespread vaccination began in the 1960s, a total of 2.6 million people per year died from measles. By 2017, global vaccination programmes had reduced this number to about 100,000 per year, most of those being children under the age of 5 years. The MMR (measles, mumps and rubella) vaccine is given to children after their first birthday, with a booster dose given at about 5 years of age.

However, pockets of unvaccinated individuals can create opportunities for outbreaks, especially for highly contagious diseases. For herd immunity to work, a large proportion of the population (roughly 75–80% depending on the pathogen) needs to be vaccinated. If these **vaccination gaps** are too large, outbreaks can occur. Throughout the world, there are sections of communities that either believe vaccines are unnecessary or that they are unsafe. **Misinformation** and **conspiracy theories** about vaccines contribute to vaccination gaps in some regions.

Go to page 134 in the **Research and Investigations Book** for an activity looking at vaccination rates.

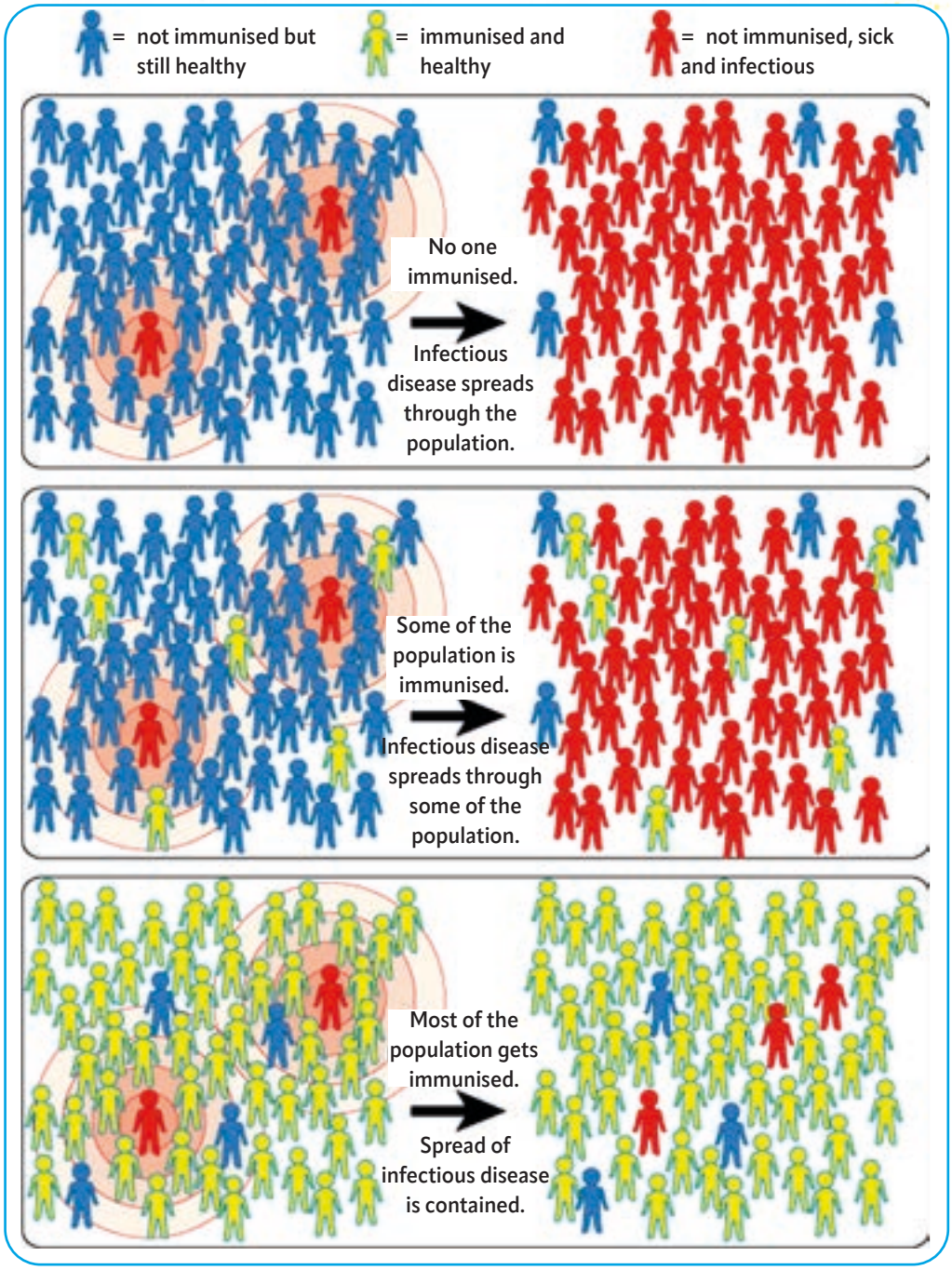


Fig. 12.30 *The importance of immunisation and herd immunity in preventing the spread of infectious (and contagious) disease.*

The Reproduction Number

The **R_0 value** is a measure of how contagious a disease is and is used to create **models** of how diseases spread within populations. It tells us the number of persons infected per person infecting. In mathematical models of infection, an $R_0 > 1$ means the infection is likely to spread in the population. When $R_0 < 1$, the infection is unlikely to spread. Diseases with a higher R_0 value are more likely to cause outbreaks: the higher the R_0, the harder it is to control the spread of disease.

Definition: The **basic reproduction number (R_0)** is the expected number of infections caused by a single infected individual, in a population where all individuals are susceptible.

The **basic reproduction number R_0** for an infectious disease allows us to estimate the proportion of immune individuals that is needed to achieve herd immunity in the population. This, in turn, tells us how many of us need to be vaccinated.

Table 12.6 *Summary of factors affecting the emergence and spread of infectious diseases.*

Factor	How it Affects Emergence and Spread of Infectious Disease
Persistence of the pathogen in a host	• Animals that harbour the pathogen without becoming ill can become **reservoirs** for the pathogen. A reservoir could provide a pool of virus that can spill over to humans and cause an outbreak. • **Long-term (chronic) infections** allow for ongoing replication of the virus, which increases the chance of mutations that could make it more transmissible or more capable of evading the immune system.
Mutations	• **Mutations** can make a pathogen more transmissible, cause more severe disease or help it evade the immune system. • The **overuse of antibiotics** (against bacteria) and **pesticides** (against insect disease vectors) can lead to the selection of resistant mutants that can become established. This makes it more difficult to treat infections and control the spread of diseases.
Immunity level present in the population	• A high number of immune individuals in a population helps protect unvaccinated individuals by reducing the likelihood of exposure to an infectious disease. This is known as **herd immunity**. • **Vaccination gaps** can create pockets of susceptible individuals who can be infected and spread the disease to others. The higher the herd immunity, the less likely that small vaccination gaps will be a problem.
Mobility in affected populations	• Regional and global **travel,** now common, can introduce pathogens into new areas, sometimes far away from the initial outbreak. • **Dense urban populations** and **large gatherings** of people can also increase the rate of transmission.
The basic reproductive number (R_0)	• **The basic reproductive number (R_0)** is the expected number of infections caused by a single infected individual. • The R_0 value is a measure of how contagious a disease is. Diseases with a higher R_0 value are more likely to cause outbreaks.

Autoimmune Diseases

An **autoimmune disease** arises when the immune system mistakenly attacks and damages healthy parts of the body. There are more than 80 different types of autoimmune disease that result from this inappropriate, overactive immune response. Cells of the immune system can attack body cells as if they are non-self.

Definition: An **autoimmune disease** arises when the immune system mistakenly attacks and damages healthy parts of the body.

Rheumatoid arthritis, multiple sclerosis (MS), type 1 diabetes, psoriasis and inflammatory bowel diseases (IBD) are some examples of autoimmune disease. These diseases are affecting more people than ever before.

Factors Affecting Autoimmune Diseases

The causes of autoimmune diseases are uncertain. Research suggests that these diseases result from interactions between genetic and environmental factors.

- **Genetics**: People with a family history of autoimmune diseases have a higher risk. Gender and ethnicity also play a role. Nearly 80% of people with chronic autoimmune disease are women. Genes can make someone more susceptible to an autoimmune disease, but do not make it a certainty.
- **Nutrition:** Some types of foods may cause inflammation in susceptible people. An imbalance of the **gut microbiome** has been linked to increased risk of autoimmune disease.
- **Pollution:** Exposure to environmental toxins such as cigarette smoke or pesticides may be a factor.
- **Infections:** Certain infections, such as COVID-19, may trigger autoimmune responses.

Potential Strategies to Reduce Cases of Autoimmune Disease

- **Understanding the interaction between genes and environment:** This could help identify individuals at high risk and raise awareness of preventative measures.
- **Dietary and lifestyle modifications:** Focusing on a healthy **microbiome**, reducing foods that are linked to inflammation, and getting enough **vitamin D** might be beneficial.
- **Early diagnosis and treatment:** Early diagnosis of autoimmune diseases can help people manage their symptoms and prevent long-term damage.
- **Immune therapies:** As our understanding of the complex interactions in the immune system increases, so too does the chance of developing personalised treatments for sufferers of different autoimmune diseases. Some **signalling proteins** produced by B cells have been shown to contribute to autoimmune diseases. There are some medications available to treat lupus and rheumatoid arthritis that target a patient's B cells to prevent the production of these signalling proteins.

Go to page 131 in the **Research and Investigations Book** for a research task on the hygiene hypothesis.

Chapter Summary

Immunity and Disease

- **Immunity** is the ability of an organism to defend itself against pathogens, cancer cells and foreign toxins.
- A **pathogen** is an organism that causes disease. A **parasite** is an organism that benefits by living in or on another organism (the host) causing it harm.
- **Infection** is the harmful colonisation of an organism by another species.
- **Antigens** are non-self substances that stimulate the production of specific antibodies and other immune responses. **Antibodies** are proteins produced by specific white blood cells in response to the presence of an antigen. An antibody will bind to a particular antigen due to its specific complementary shape.

Viruses

Viral Replication

- **Attachment:** the virus attaches to the host cell surface.
- **Insertion:** the viral DNA or RNA enters the host cell.
- **Biosynthesis:** viral nucleic acid and protein molecules are made using the host's cellular machinery.
- **Assembly:** new viral particles are assembled, typically composed of nucleic acid in a protein coat (capsid).
- **Release:** the cell lyses, releasing newly synthesised viral particles, or virus particles exit the cell by budding, gaining a lipid envelope.

Mode of Action

- DNA viruses typically replicate in the nucleus, use the host cell DNA polymerase, are more stable, and have lower mutation rates.
- RNA viruses typically replicate in the cytoplasm, contain their own RNA polymerase, are less stable and have a higher mutation rate than DNA viruses. Immunity is usually short-lived and they are more difficult to vaccinate against than DNA viruses.

Innate Immune System

- The **innate immune system** is a non-specific defence system composed of barriers to entry and first lines of protection against pathogens.
  - Skin is a **physical barrier**. **Sebum** contains antimicrobials and moisturises, maintaining the integrity of the skin.
  - **Hydrochloric acid** in the stomach kills microorganisms.
  - **Mucus** and **cilia** in the trachea and bronchi trap pathogens.
  - Damaged tissue releases **histamine** which raises the temperature and increases blood flow to the wound. **Clotting** by platelets seals wounds.
  - **Monocytes** are large white blood cells that are activated to become phagocytic **macrophages** which engulf and destroy pathogens and infected cells.
  - **Natural killer cells** are lymphocytes that destroy virus-infected and tumour cells.

HL The **adaptive immune** system is composed of specialised cells and processes that protect against specific pathogens.

HL B lymphocytes

- B lymphocytes (B cells) are produced and mature in the red bone marrow before they migrate in the lymph and the blood.
- Activated by T cells and **antigen-presenting** macrophages to divide rapidly.
- **Plasma cells** are activated B cells that release their **B cell receptor** proteins in the form of **antibodies**.
- Plasma cells produce large amounts of antibody molecules.
- Memory B cells continue to circulate post-recovery for rapid response in case of re-infection.

HL T lymphocytes

- T lymphocytes (T cells) are produced in the red bone marrow but mature in the thymus.
- T cells are activated by **antigen-presenting** macrophages or B cells.
- **Killer T cells** kill damaged or virus-infected cells and cancer cells.
- **Helper T cells** coordinate the immune response by activating other immune cells.
- **Suppressor T cells** regulate the immune system, inhibiting other immune cells once the infection is under control.

- **Memory T cells** can live for many years after initial infection, allowing for a quick immune response in the case of a secondary infection.

- **Acquired immunity** may be **active** or **passive**.
- **Active immunity** is acquired when our body's own immune cells produce antibodies in response to disease.
  - **Natural active immunity** occurs when a person encounters an infection and the immune system responds and overcomes the pathogen.
  - **Artificial active immunity** occurs when a person is given an inactivated pathogen (or a part of the pathogen). This is known as **vaccination**.
- **Passive immunity** is acquired when a person receives antibodies made by another organism.
  - **Natural passive immunity** is acquired by a foetus when maternal antibodies are transferred through the **placenta,** or by a baby in **breast milk** and colostrum.
  - **Artificial passive immunity** occurs when antibodies specific to a pathogen or toxin are obtained from the blood of an immune animal.

Disease

- **Prevention** of infectious diseases:
  - Immunisation (vaccination).
  - Sanitation and hygiene.
  - Vector control.
  - Education.
- **Treatment** of infectious diseases:
  - Antibiotics.
  - Antivirals.
  - Antifungals.
  - Antiparasitics.
- An **emerging infectious disease** is one that has only recently appeared in a population or is rapidly increasing in certain parts of the world.
- Factors affecting the spread and emergence of infectious disease (see Table 12.6 for detailed summary):
  - **Persistence of the pathogen:** animal reservoirs, asymptomatic individuals.
  - **Pathogen mutation and adaptation:** transmissibility, virulence, immune system evasion.
  - **Movement of organisms:** urban commuting, air travel, global trade, climate change.
  - **Antibiotic or chemical resistance:** antibiotic-resistant bacteria, insecticide resistance.
  - **Immunity level** present in the population: herd immunity and vaccination gaps.
  - **The reproduction numbe**r (R_0): number of persons infected per person infecting.
- **Autoimmune diseases:** An **autoimmune disease** arises when the immune system mistakenly attacks and damages healthy parts of the body, e.g. type 1 diabetes, rheumatoid arthritis, psoriasis, lupus, inflammatory bowel disease.
- **Factors** affecting autoimmune diseases:
  - **Genetics:** family history, gender, ethnicity.
  - **Nutrition:** e.g. foods that can increase or decrease inflammation, microbiome imbalance.
  - **Pollution**: e.g. cigarette smoke or pesticide**.**
  - **Infections**: e.g. COVID-19 infection.
- **Strategies to reduce cases** of autoimmune disease:
  - Understanding the interaction between genes and environment**.**
  - Dietary and lifestyle modifications**.**
  - Early diagnosis and treatment. Immune therapies.

Questions

1. (a) Explain what is meant by the term *immunity*.
(b) What is an infection?
(c) Distinguish between a *pathogen* and a *parasite*.
(d) Explain the term *obligate parasite*.
(e) Give two examples of obligate parasites.

2. (a) Distinguish between the terms *infectious* and *contagious* in relation to disease.
(b) Name a disease that is caused by an microscopic organism from the kingdom Protista.
(c) Give one example of a parasitic animal.
(d) What is meant by the term *host*, in relation to parasites?
(e) What is a prion?
(f) Give one example of a disease caused by a prion.
(g) What makes prions unusual as pathogens?

3. (a) State what is meant by the term *antigen*.
(b) Name two classes of biomolecules that could be antigens.
(c) Define the term *antibody*.
(d) Name the class of biomolecule that antibodies belong to.
(e) Briefly describe antigen–antibody interactions, using a labelled diagram to illustrate your answer.

4. (a) Distinguish between the *innate* and *adaptive* immune responses.
(b) State two ways in which the skin prevents entry of pathogens.
(c) Describe how mucus and cilia in the breathing system act to protect the body from pathogens.
(d) Outline how stomach acid has an immune function.

HL **5.** Write a detailed account of the role of monocytes in the immune response. In your answer, refer to macrophages, phagocytosis and antigen-presentation.

HL **6.** (a) What kind of white blood cells are natural killer (NK) cells?
(b) What kind of cells do NK cells target?
(c) What distinguishes NK cells from T cells?
(d) Describe how NK cells act to protect the body from pathogens.
(e) NK cells are important in the innate immune response. Explain how their activity also links with the adaptive immune system.

HL **7.** (a) In what part of the body would you expect to find relatively large numbers of B cells?
(b) What is a B cell receptor?
(c) Describe how B cells are activated.
(d) What is the role of a plasma cell?
(e) What is the role of a memory B cell?

HL **8.** (a) Describe how T cells are activated.
(b) Outline the role of helper T cells in B cell activation.
(c) State one other role of helper T cells.
(d) State the role of killer T cells.
(e) Explain how killer T cells carry out their role.
(f) What is the function of suppressor T cells?

HL **9.** (a) Explain the term *immunological memory*.
(b) Sketch a graph to show the difference between the primary and the secondary immune responses.
(c) Outline the activity of memory B cells and memory T cells in the secondary immune response.

HL **10.** (a) What is meant by *antigen-presentation*?
(b) Name one type of antigen-presenting cell.
(c) Outline the response of a helper T cell when it encounters an antigen-presenting cell.
(d) Explain how antigen-presentation can be considered as a link between the innate and the adaptive immune systems.
(e) Explain how killer T cells recognise cancer cells and infected body cells.

HL **11.** Helper T cells are sometimes said to be the most important cells of the immune system. Do you think this is justified? Evaluate the statement by writing a detailed account of the roles and activity of helper T cells.

HL **12.** There are many ways in which lymphocytes interact with one another and with other cells of the immune system. Outline these lymphocyte interactions and evaluate the importance of each one.

13. **(a)** Distinguish between *active* and *passive acquired immunity*.

(b) Explain how active immunity can be acquired naturally.

(c) What is meant by *vaccination*?

(d) Outline how vaccination gives artificial active immunity to an individual.

(e) Describe how natural passive immunity is acquired.

(f) What is meant by the term *immunisation*?

(g) Explain how artificial passive immunity can be used to immunise an individual.

14. **(a)** List three public health measures that are in place to prevent the spread of infectious diseases in society.

(b) Explain the role of education in reducing the spread of infectious diseases.

(c) What does the abbreviation WHO stand for?

(d) Explain the role of the WHO in preventing the spread of infectious diseases.

15. **(a)** What is an antibiotic?

(b) Explain why antibiotic resistance has become a global problem in controlling infectious disease.

(c) Give one reason for the spread of antibiotic resistance.

(d) Suggest one solution to the problem of antibiotic resistance in bacterial populations.

16. **(a)** Explain why antibiotics are not suitable to treat viral or fungal diseases.

(b) What is an antiviral?

(c) Name two diseases that can be treated with antivirals.

(d) Name two diseases that can be treated with antifungal preparations.

(e) What is an antiparasitic?

17. Write an account of the factors affecting the spread and emergence of infectious disease under three sub-headings: Pathogen persistence and adaptation; Mobility; Herd immunity.

18. **(a)** What is an autoimmune disease?

(b) Give three examples of common autoimmune diseases.

(c) Genetics appears to play a role in the risk of developing an autoimmune disease in some cases at least. Suggest how genetics may play a role.

(d) List two environmental factors that are associated with increased risk of being affected by an autoimmune disease, and explain how this might occur.

(e) Outline two ways in which lifestyle changes might reduce the risk of developing an autoimmune disease.

19. **(a)** What is ash dieback disease?

(b) What kind of organism causes the disease?

(c) Name another plant disease that has affected Irish woodlands.

(d) Suggest one way that these invasive plant pathogens could have arrived in Ireland.

(e) Explain why these non-native pathogens have caused so much destruction to native Irish trees.

(f) Suggest one measure by which the introduction of non-native pathogens into Ireland could be limited.

CHAPTER 21

Biotechnology and Genetic Engineering

At the end of this chapter you should be able to:

- Outline the concept of genetic engineering and the process involved.
- Describe the applications of genetic engineering.
- Outline what is meant by DNA profiling and describe its potential uses.
- HL Model the steps involved in generating a DNA profile.
- Investigate patterns using a DNA profile.
- HL Outline the principle of DNA sequencing.
- Use a genome database to search for alleles that are known to cause specific genetic diseases.
- Appreciate the value of technology in analysing large quantities of genetic information.
- Describe the use of genetic data.
- Outline the use of genetic modification, DNA testing and stem cells.
- Discuss the ethical issues arising from advancements in genetic technologies.

To help you understand this chapter, remember:

- The structures of prokaryotic cells (Chapter 4 and Chapter 19).
- The structure of DNA (Chapter 3).
- Gene expression is the process by which information in a gene is used to make a specific protein (Chapter 3).
- The process of DNA replication (Chapter 10).

In this chapter you will learn how knowledge of biology and innovation have led to the development of new forms of biotechnology, such as genetic engineering, DNA sequencing and bioinformatics. You will build on your learning about microorganisms to understand the roles they have played in many advances in biotechnology. You will appreciate the many applications of these modern biotechnology processes, including in the fields of medicine, agriculture and industry.

Genetic Engineering

Biotechnology is a broad term for the use of the processes of living things, or their products, to meet the needs of humans. Using yeast to bake bread, **enzymes** to produce lactose-free milk or bacteria to produce yoghurt are all types of biotechnology. **Genetic engineering** is a type of biotechnology that involves altering the **DNA** of an organism. The applications of genetic engineering are wide-ranging and will be discussed later in this chapter.

Definition: Biotechnology is the use of organisms or biological systems to make products or carry out processes that are useful.

Definition: Genetic engineering is the manipulation of the DNA of an organism.

Tools Used in Genetic Engineering

Many of the tools used in genetic engineering were discovered as naturally occurring components of organisms. They were isolated from those organisms and in most cases modified for use in the laboratory. Three of the most important tools are restriction enzymes, DNA ligase and plasmids.

Restriction Enzymes

Restriction enzymes were first discovered in the 1950s. They are part of a defence mechanism used by bacteria to protect themselves from viral infection. These naturally occurring enzymes chop up the DNA of viruses that infect the bacterial cell, so that the virus cannot replicate (see Chapter 12). Restriction enzymes are usually named after the bacterium they were isolated from. A commonly used enzyme is *Eco*RI, which was isolated from the bacterium *E. coli*.

Each restriction enzyme cuts DNA at a specific base sequence, usually around 6–8 DNA base pairs in length (Fig. 21.1). Often the cut will produce an overhang of single stranded DNA. If two pieces of DNA are cut with the same enzyme, the complementary bases in these regions, known as 'sticky ends', will form hydrogen bonds with each other. In this way DNA fragments from different organisms can be joined together. There are over 600 different restriction enzymes commercially available today, so biologists have great flexibility in where they can cut (and insert) when manipulating DNA molecules.

Definition: A restriction enzyme is an enzyme that cuts the DNA molecule at a specific base sequence.

Link 21.1

Scan this code to watch an animation of a restriction enzyme cutting a DNA molecule.

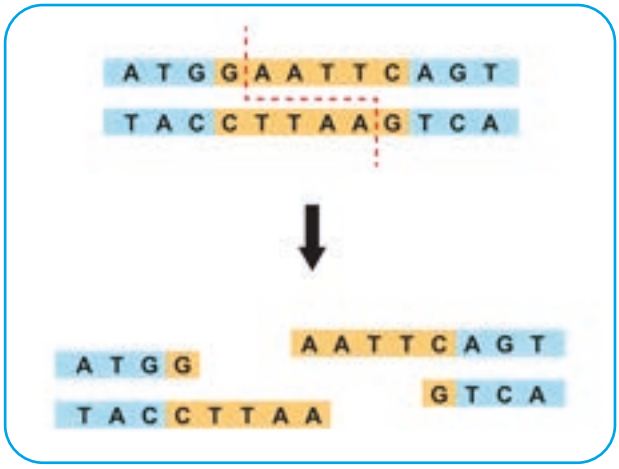


Fig. 21.1 *The recognition site for the restriction enzyme* Eco*RI is shown in orange. The enzyme cuts between the G and the A on each strand (indicated by the red dotted line) leaving sticky ends when the DNA is cut.*

DNA Ligase

Definition: **DNA ligase** is an enzyme that forms bonds between the sugar and the phosphate group of adjacent nucleotides in a DNA molecule.

DNA ligase was discovered in 1967. It is an enzyme that forms bonds between the sugar and the phosphate group of adjacent nucleotides in a DNA molecule. Ligase plays a role in **DNA replication**. It is also involved in DNA repair mechanisms that are crucial to the **cell cycle** checkpoints (Chapter 10). In genetic engineering, DNA ligase is used to join fragments of DNA. While the complementary bases will form hydrogen bonds when sticky ends are brought together, DNA ligase joins the sugar phosphate backbones of both DNA molecules.

Plasmids

Definition: A **plasmid** is a small circular DNA molecule found in prokaryotic cells that often contains antibiotic resistance genes.

We saw in Chapter 4 and Chapter 19 that **prokaryotic** cells have two types of DNA molecule in their cells. Along with chromosomal DNA they can also contain small circular molecules of DNA called **plasmids**. Plasmids often carry **antibiotic** resistance genes encoding proteins that protect the bacterial cell from the effects of antibiotics. Plasmids often move between bacterial cells in nature, so early genetic engineers realised that they could be very useful in biotechnology to carry a gene of interest into a bacterium.

While these small molecules of DNA occur naturally in bacteria, they have been modified for use in genetic engineering. One of the main modifications is the inclusion of sites for known restriction enzymes (Fig. 21.2). This allows genes to be easily inserted into the plasmid.

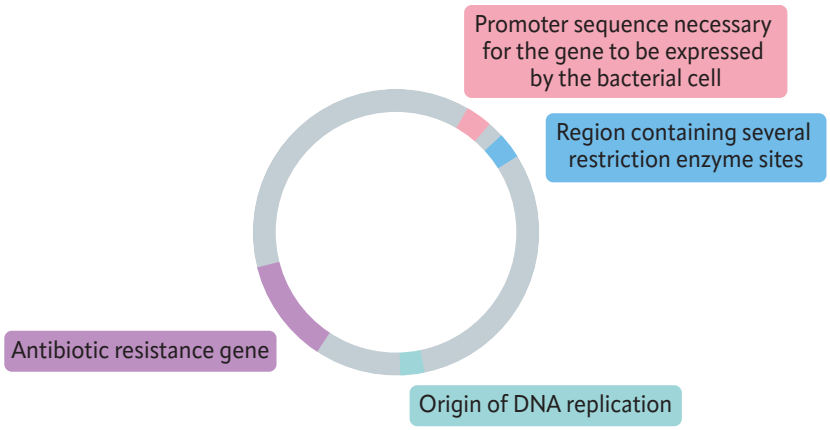


Fig. 21.2 *Plasmids are small circular DNA molecules. They have been modified to include restriction enzyme sites for use in genetic engineering.*

Plasmids also contain an origin of replication, which means the molecule will be copied by the bacterial cell's DNA replication machinery. They may also have a **promoter** sequence just before the site of gene insertion. This sequence is recognised by **RNA polymerase** so that a gene inserted into the plasmid will be expressed by the host cell.

The Process of Genetic Engineering

Genetic engineering involves inserting a gene of interest into a plasmid and then introducing the plasmid back into bacterial cells. The process of genetic engineering is also known as **gene cloning**. A clone is an exact copy of something. In this process many copies of a particular gene of interest are made.

At its simplest, the process (summarised in Fig. 21.5) involves four steps:

1. Isolation of DNA.
2. Cutting of DNA with restriction enzymes.
3. Ligation.
4. Transformation and expression.

1 Isolation of DNA

The DNA is isolated from cells. The **plasmid DNA** is isolated from bacterial cells. While plasmids are commonly used to carry the gene of interest, other mechanisms have been used. For example, some viruses have been used to carry genes of interest into other organisms in genetic engineering. Whatever is used to carry the gene of interest into the new organism is called a **vector**. Plasmids are often referred to as cloning vectors.

The DNA containing the **gene of interest** is isolated from the appropriate organism.

Isolation of DNA involves breaking open the cell membranes and separating the DNA from the other parts of the cell and the proteins found in chromosomes.

2 Cutting of DNA

Once both DNA samples have been isolated, they are cut with the same restriction enzyme. Using the same enzyme means that they will have the same sticky ends, so the gene of interest can be easily inserted into the plasmid DNA (Fig. 21.3).

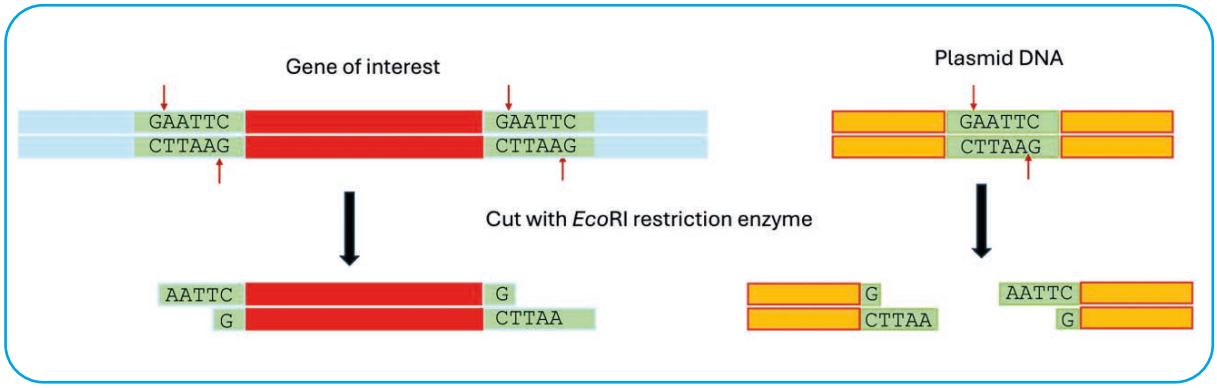


Fig. 21.3 *DNA containing the gene of interest and the plasmid DNA are both cut with the same enzyme. In the example here,* EcoRI *is used to cut both DNA molecules.*

3 Ligation

Definition: A **recombinant** plasmid is a plasmid containing a fragment of foreign DNA.

The DNA molecules are mixed together and the sticky ends with complementary bases form weak hydrogen bonds. Ligation is the process of using DNA ligase to form the bonds in the sugar phosphate backbone between the two DNA molecules. In this way, the gene of interest and the plasmid DNA are strongly joined together (Fig. 21.4). The plasmid containing the gene of interest is known as a **recombinant plasmid**, meaning DNA from two different sources has been combined.

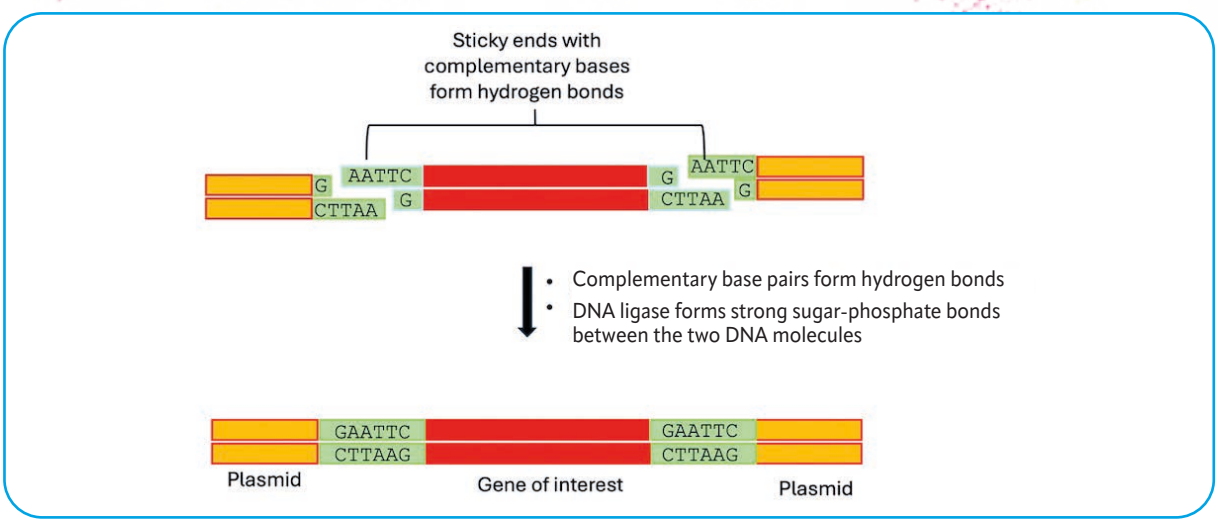


Fig. 21.4 *The DNA molecules are mixed together and the sticky ends with complementary bases form hydrogen bonds. Ligase is added to form the bonds in the sugar phosphate backbone of adjacent nucleotides.*

4 Transformation and Expression

Definition:
Transformation is a process where bacteria take up DNA from their environment.

Transformation

The recombinant plasmid is reintroduced into bacterial cells by a process called **transformation** (Fig. 21.5). Bacterial transformation is a process where bacteria take up DNA from their environment.

Both the cell membrane of bacterial cells and the DNA molecule are negatively charged so they tend to repel each other. The bacteria are treated with a chemical that neutralises their negative charge. This is followed by a heat-shock treatment, which creates pores in the cell membranes, allowing recombinant plasmid molecules to enter the bacterial cells. They are then grown in optimal conditions to allow them to recover.

Expression

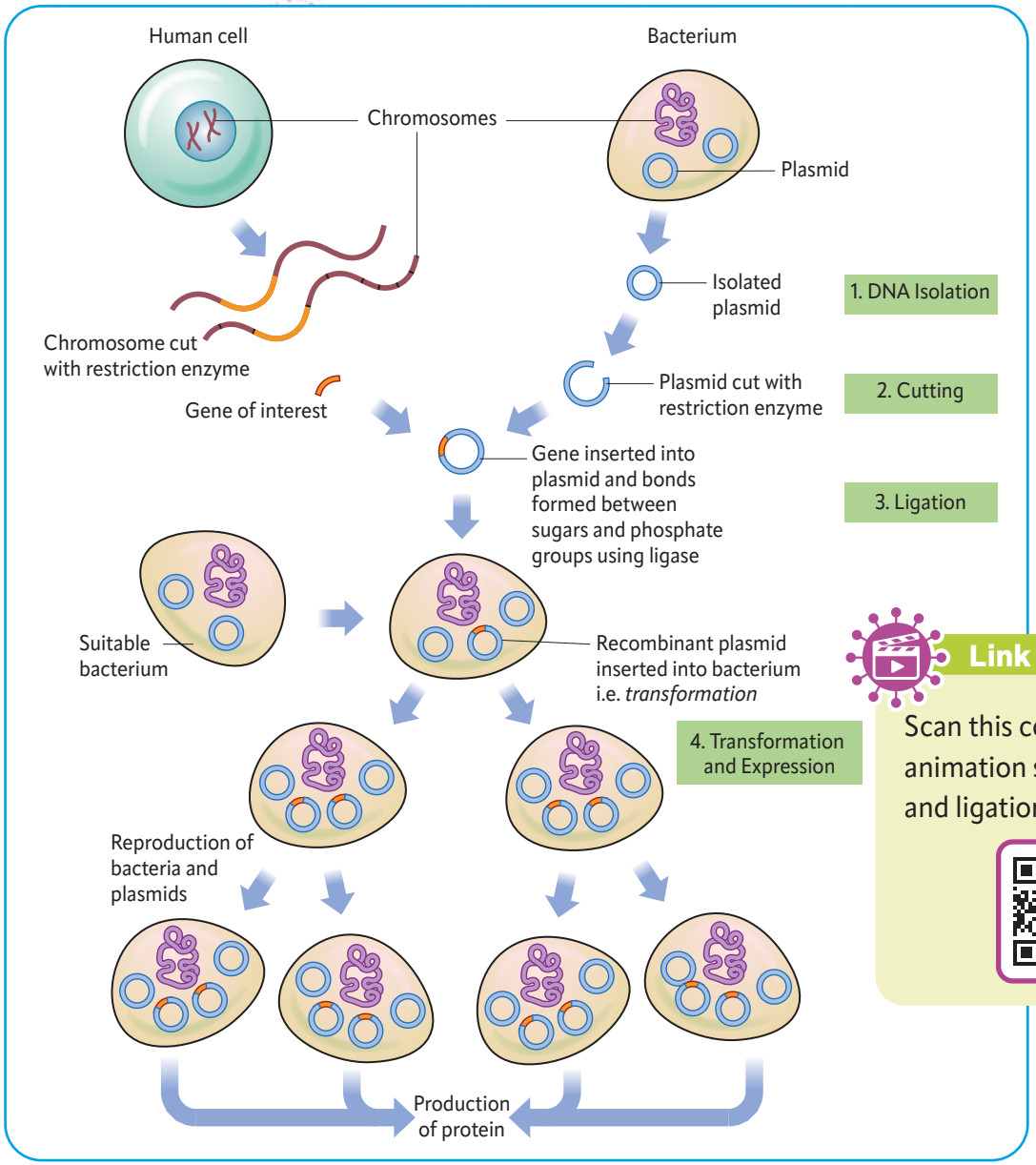
Gene expression is the final stage of the process, where the information in the gene of interest is used to produce a protein. In genetic engineering, the gene may be expressed in the bacterial cells or in cells of another organism. Regardless of where expression takes place, large numbers of bacterial cells containing the plasmid are grown.

Once the transformation process has been carried out the bacterial cells are grown on agar plates. As the plasmid includes an antibiotic resistance gene, this antibiotic can be included in the agar. This means that only bacterial cells that have taken up the plasmid during transformation will grow.

When the bacterial colonies have grown on the agar plates, they can then be picked and placed in flasks of liquid medium, so that large numbers of cells can be obtained (Fig. 21.6).

Sometimes the bacterial cells are used to make the protein encoded by the gene. If this is the case, the cells are grown in a **bioreactor** (Chapter 19) and the expressed protein is isolated.

In other applications, the plasmid is isolated from the cells grown in liquid culture. By growing large numbers of the cells, a large supply of the recombinant plasmid DNA is obtained. It can then be used in other applications (see opposite).



Link 21.2

Scan this code to watch an animation showing cutting and ligation steps.

Fig. 21.5 *The process of genetic engineering.*

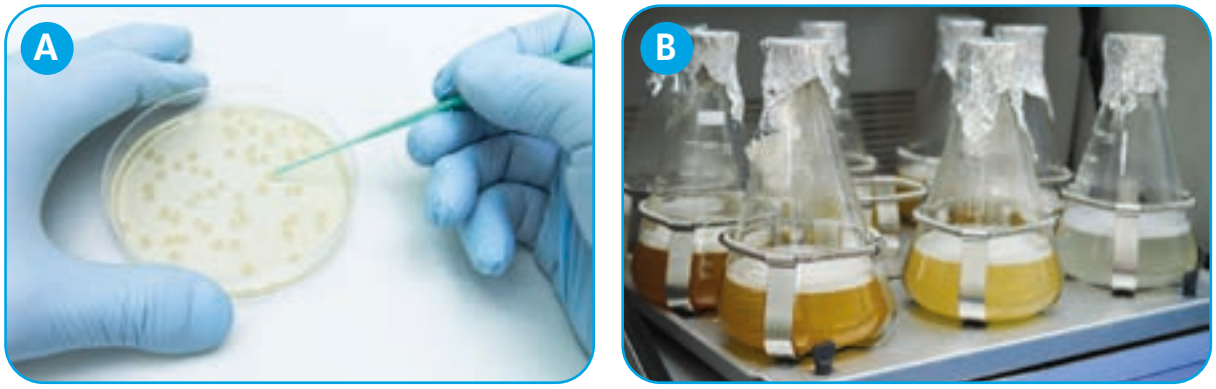


Fig. 21.6 *(A) Once bacterial colonies have formed on the agar plate they are picked and placed into liquid medium. (B) Flasks of liquid medium are grown to produce a large number of cells containing the recombinant plasmid. This can be further scaled up depending on how much is required.*

Go to pages 210 and 212 in the **Research and Investigations Book** for activities on genetic engineering involving DNA isolation and cutting.

Applications of Genetic Engineering

The applications of genetic engineering are wide ranging. Some examples are described below.

Medicine

Genetic engineering can be used to produce proteins in bacterial cells once the cells have been transformed with the recombinant plasmid. One of the first genes to be expressed in bacterial cells was the gene for human **insulin**. Before the 1980s, people with diabetes were treated with insulin from cows or pigs. The production of human insulin by bacterial cells not only provided a human form of the protein but also enabled large scale production, increasing the supply of this life-saving hormone. Other proteins used to treat medical conditions have been produced in a similar way.

Gene cloning is also routinely used in medical research. It can be used to develop vaccines and cancer therapies, as well as helping to develop our understanding of the role of certain proteins or structures within a cell or organism. For example, the gene for a fluorescent protein, such as green fluorescent protein (GFP), can be introduced into an organism or cell. When GFP is expressed, it will glow green under certain wavelengths of light. If the fluorescent protein is joined to structures in a cell, scientists can develop a greater understanding of the role these structures play in the cell or organism. This can lead to the development of new therapies (Fig. 21.7).

Fig. 21.7 *Genetically modified mosquito larvae. The gene for green fluorescent protein (GFP) has been introduced into the mosquito genome. The green glow shows that the GFP gene has been successfully introduced. This raises hope that a gene could be introduced that would make the mosquitoes unable to carry the parasite that causes malaria, which would save millions of lives.*

Agriculture

Rubisco is a plant enzyme that catalyses part of the light-independent stage of **photosynthesis**. The version of the enzyme produced by some cyanobacteria is more efficient than that of plants. By inserting the gene for rubisco from cyanobacteria into plants, their rate of photosynthesis can be increased. Researchers have successfully expressed the cyanobacterial enzyme in test plants, but the technology is still in the early stages of development.

Cotton is an economically important crop that can be damaged by insect pests (Fig. 21.8). One species of bacterium (*Bacillus thuringiensis*, abbreviated as BT) produces crystals of a protein that is toxic to specific insect larvae that eat the cotton plant. The gene for the toxin has been introduced into cotton plants (known as BT cotton), so that when the pests eat the cotton they die. This prevents damage to the crops and reduces the use of chemical pesticides.

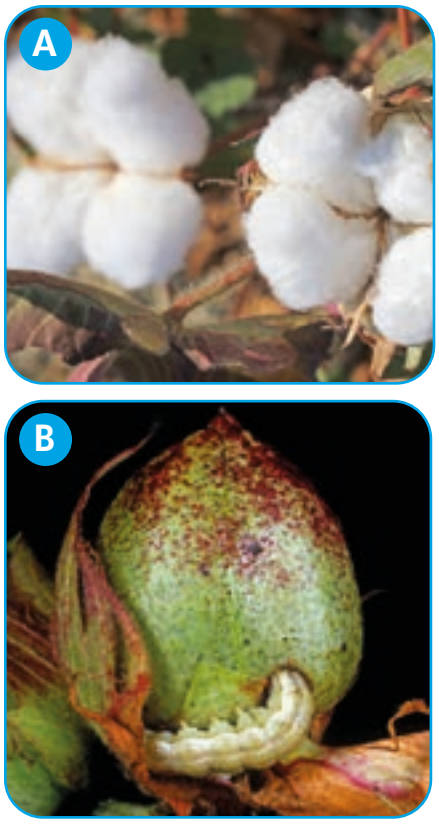


Fig. 21.8 *(A) Cotton fibres develop within the fruit of the cotton plant. As the fruit ripens it turns brown. The developing cotton fibres continue to expand until the fruit splits open. (B) The cotton bollworm (*Helicoverpa zea*) caterpillar feeding on green unripe fruit of the cotton plant.*

Industry

Enzymes are important for many industrial applications, in particular in the textile, paper and food industries (Chapter 7). Regardless of the original source of the enzyme, genetic engineering can be used to create microorganisms that will produce any enzyme. The use of microorganisms has a number of advantages. In many cases the enzyme produced will be released from the cell, making the purification process easier. The rapid reproduction rate of microorganisms means a high yield of product is rapidly obtained. The process also enables modification to produce improved characteristics and higher yields of the final protein.

Genetic Modification

A **genetically modified** (GM) organism is one in which the DNA has been altered in a way that does not occur naturally.

Definition: A **genetically modified** organism is an organism that has been altered using genetic engineering techniques.

While traditional genetic engineering techniques have been used to genetically modify organisms, newer methods are developing all the time. One such example is gene editing using CRISPR-Cas9. This method offers a more efficient means of modifying DNA. It can be used to introduce small changes or to remove or replace a gene altogether. Using this technique, multiple gene edits can be carried out quickly and simultaneously.

Extend Your Knowledge

Scan this code to learn more about CRISPR-Cas9.

Go to page 216 in the **Research and Investigations Book** for a research task on the use of genetically modified organisms in agriculture.

Go to page 218 in the **Research and Investigations Book** for an activity on modifying a DNA sequence.

HL The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a molecular biology technique used to make millions of copies of a particular section of DNA. It was invented in 1983 by Kary Mullis, a US biochemist who was awarded a Nobel Prize in 1993. It has revolutionised molecular biology.

Definition: The **polymerase chain reaction** is a process used to make many copies of a particular section of DNA.

PCR is based on the process of **DNA replication**. The first step in DNA replication involves separation of the two strands in the double-stranded DNA molecule. Each of the two strands then acts as a **template**. Free nucleotides bind to their complementary base pairs on the exposed template strands, and two new strands of DNA are synthesised by the enzyme **DNA polymerase**.

In PCR, instead of happening in a cell, the process occurs in solution in a small tube that is placed into a laboratory instrument called a **PCR machine**. The DNA polymerase used in PCR is ***Taq* polymerase**, a heat-stable **enzyme** that was originally isolated from the **thermophile** *Thermus aquaticus*. This enzyme has an optimal temperature of 72°C but, importantly, is not **denatured** during the high temperatures needed for one part of the PCR.

The Components of PCR

- **DNA template**: a sample of the DNA containing the region to be copied.
- **DNA nucleotides**: a supply of each of the four nucleotides (A, T, G and C).
- **DNA primers**: these are two short single-stranded DNA molecules, designed and synthesised to have a specific DNA sequence. One of the primers is designed to be complementary to one end of the target region on one of the template strands, while the other primer is complementary to the other end of the target region on the opposite template DNA strand (Fig. 21.9).
- **PCR buffer**: a solution that maintains the pH.

Link 21.3

Scan this code to watch an interactive animation on the polymerase chain reaction.

Link 21.4

Scan this code to watch a video animation of the polymerase chain reaction.

Link 21.5

Scan this code to watch an animation showing how PCR amplifies small samples of DNA.

The Process of PCR

1 Denaturation

Similar to DNA replication, the first step involves separation of the two strands of the double-stranded DNA molecule (Fig. 21.9). This is done by increasing the temperature to above 90°C. Exposure to this temperature for even 30 seconds is enough to break the **hydrogen bonds** between the complementary bases, allowing the two strands to separate. These two strands both become **templates** for the primers to bind to.

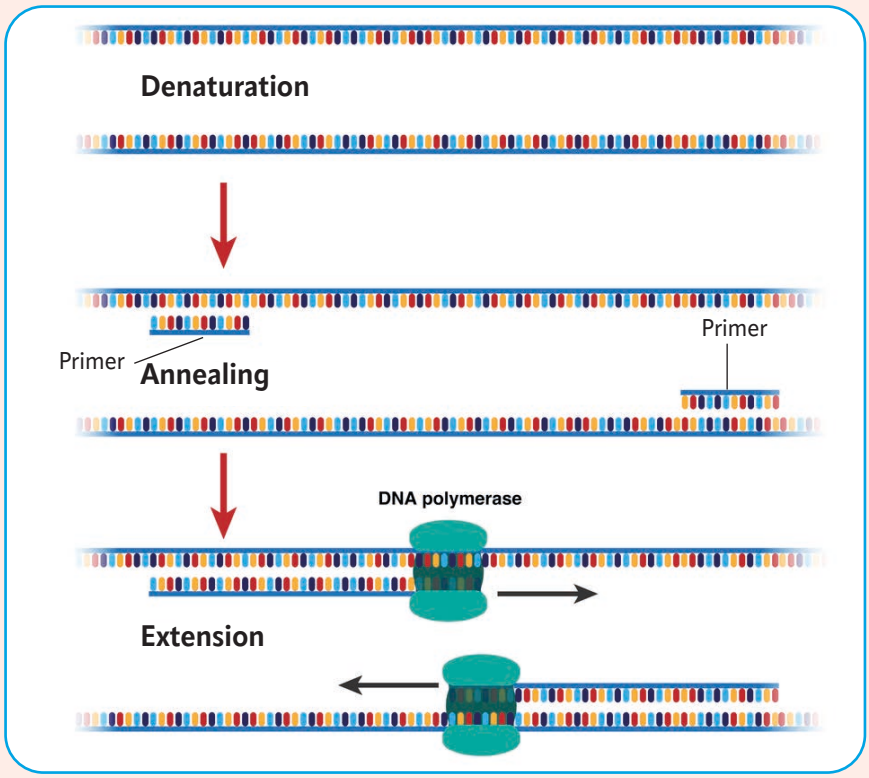


Fig. 21.9 *The process of PCR.*

2 Annealing

The temperature is lowered, typically to between 45°C and 60°C, which allows the **DNA primers** to bind by forming hydrogen bonds with **complementary** sequences. One primer has been designed to bind at one end of one of the template strands, while the other is complementary to the opposite template strand at the other end of the target sequence.

3 Extension

***Taq* polymerase** adds free nucleotides to their complementary bases from the ends of each primer, synthesising two new strands in opposite directions (Fig. 21.9). These new strands are complementary to the two template strands. This step usually takes less than a minute to complete.

This process of denaturation, annealing and extension is then repeated over and over. When one cycle of PCR is complete there are two-double stranded molecules of the target region of DNA (Fig. 21.10). Just as in cellular DNA replication, one strand is from the original molecule, and one is newly synthesised. These DNA molecules serve as templates for the next cycle of PCR.

In an ideal PCR, the number of DNA molecules doubles in each cycle. Between 25 and 35 cycles are usually completed in a single PCR, which usually results in up to a billion copies of target DNA.

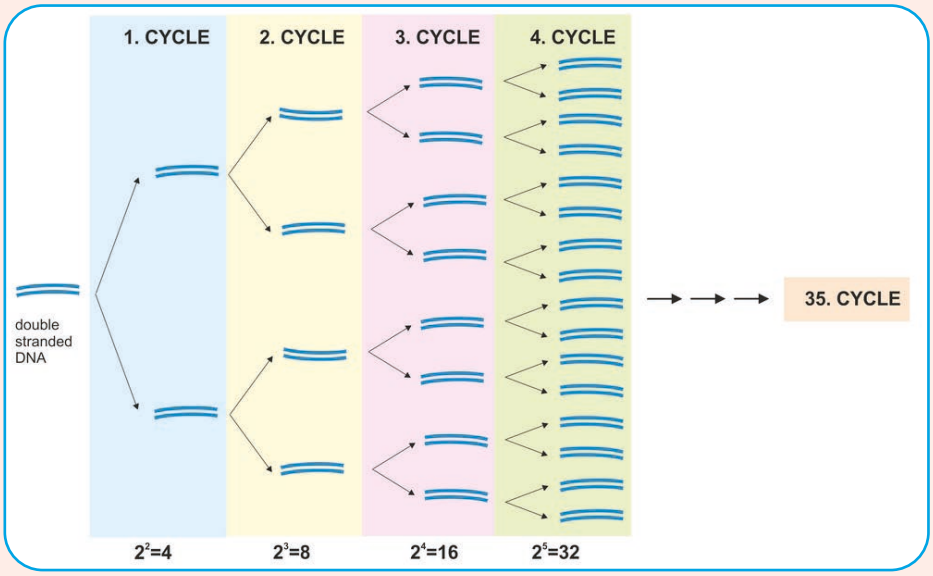


Fig. 21.10 *A huge number of copies of the region of interest are made during PCR. After 35 cycles, around 1 billion molecules of the target region can be produced.*

Applications of PCR

PCR has many applications. As we will see below, it is used in the preparation of a DNA profile.

During the COVID-19 pandemic it was used to test for the presence of the SARS-CoV-2 virus. The primers used were specific to a region of the virus's genetic material. A positive COVID-19 test confirms the presence of the virus. However, if the test is negative it could be that there is no virus present or that the levels in the sample are too low to detect.

Modern genetic engineering techniques have incorporated PCR into the process. There are several advantages to using PCR to make copies of a gene of interest, rather than cutting it out of a DNA molecule using restriction enzymes:

- PCR requires only a tiny amount of template DNA.
- PCR makes it easier to obtain large amounts of the gene of interest, compared to using restriction enzyme digests.
- Restriction enzyme sites can be included in the primer sequences, which means that regardless of what sites naturally occur either side of the gene of interest it can still be inserted into a plasmid.
- PCR also allows the introduction of base changes in the section of DNA being copied.

DNA Profiling

DNA profiling is a process used to identify individuals based on their unique genetic profile. All human beings are 99.9% genetically identical. However, in the 0.1% of our DNA that is different, there are certain regions that are highly variable in length. A person's DNA profile is prepared by separating fragments of their DNA based on size. This can then be compared to other profiles.

Definition: DNA profiling is the process of making a unique pattern of bands from a person's DNA that can be distinguished from the pattern of another person.

Each person's profile is unique, unless they are identical twins. Individuals from the same family will share some of the same sized fragments. A child will inherit a mix of their parents' variable regions.

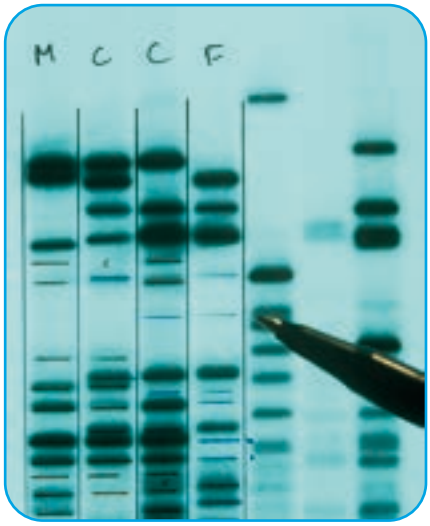


Fig. 21.11 *DNA profiles of a mother (M), father (F) and two of their children (C).*

Applications of DNA Profiling

DNA profiling is most commonly used in forensics and to identify family relationships.

Identifying Family Relationships

As children inherit their DNA from their parents, their profiles will include some fragments that are the same size as those of their father and some that are the same size as those of their mother. By comparing DNA profiles, parental relationships can be determined. A child's DNA profile should be a combination of both parents' (Fig. 21.11).

Forensics

The term **forensics** refers to the use of scientific techniques to solve crimes. DNA profiling can be used to determine the source of DNA found at a crime scene. By comparing profiles of DNA evidence with that of any suspects it can confirm whether suspects were present at the scene (Fig. 21.12). A profile match does not prove guilt, it only suggests that the person may have been at the crime scene.

DNA profiling was first used in 1986 and has since become a powerful tool in forensics, leading to many convictions being overturned on the basis of DNA evidence.

In 1998 the Federal Bureau of Investigation (FBI) in the United States identified 13 different chromosome regions it would use to identify individuals. In 2017 they extended that number to 20.

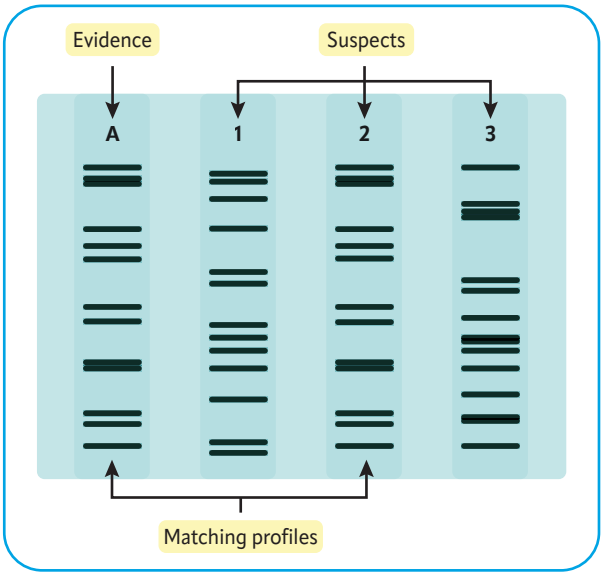


Fig. 21.12 *In forensics, DNA profiles of suspects can be prepared and compared to profiles of DNA samples found at a crime scene. Here, suspect number 2 has the same profile as the evidence, which suggests that they were present at the crime scene.*

This means that they look at 20 different variable regions in a person's DNA to construct a person's DNA profile (Fig. 21.13).

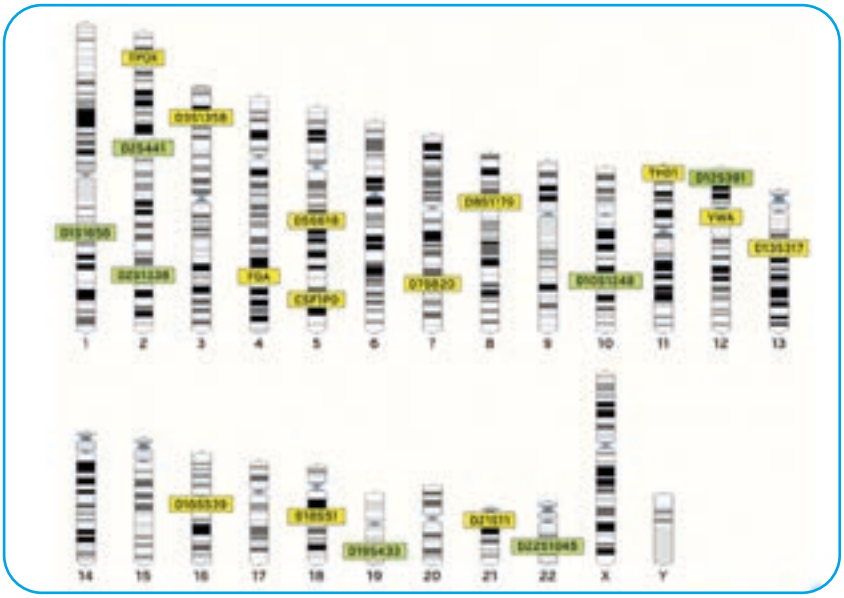


Fig. 21.13 *The chromosome locations of the variable regions used by the FBI to create a DNA profile. The original 13 are highlighted in yellow, and the seven added in 2017 are highlighted in green. © Ensembl (www.ensembl.org)*

Go to page 219 in the **Research and Investigations Book** for an activity on DNA profiling.

HL Generating a DNA Profile

The variable regions most commonly used in DNA profiling are called **short tandem repeats** (STRs). These are short sequences of DNA, usually about 2–5 bases, that are repeated. The number of times the sequence is repeated will vary from person to person. The DNA profiles of two people show the differences between the sections of DNA containing these repeats.

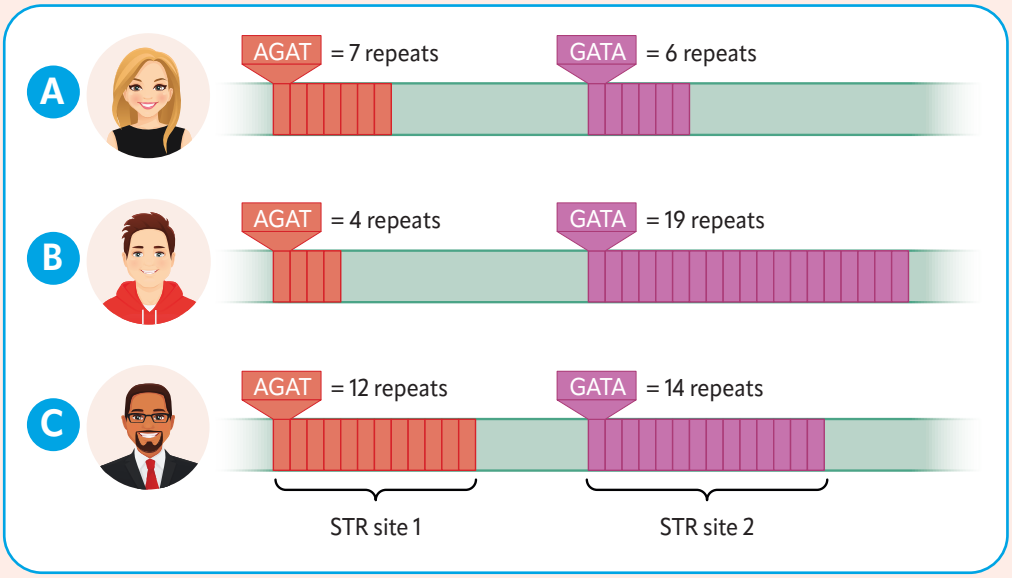


Fig. 21.14 *The number of repeats in each STR will vary from person to person. In person A, the sequence AGAT is repeated 7 times, while in person B it is repeated 4 times and in person C it is repeated 12 times.*

1 Extracting DNA

The first step involves extracting or isolating DNA from the person's cells. The most convenient way to do this is to take a cheek swab. This method is similar to that used to obtain cheek cells for viewing under the microscope. A sterile cotton swab is rubbed on the inside of the person's cheek and then placed in a solution to prevent it from drying out.

Other types of samples can also be used to prepare a profile. For example, blood cells, hair follicles and semen can also provide DNA. The DNA isolation method is the same as that used for the process of genetic engineering.

2 The Polymerase Chain Reaction (PCR)

DNA primers are designed that are complementary to the sequences of DNA at the boundaries of each STR region. As a result, the PCR produces a number of different sized DNA fragments corresponding to each STR region.

Fig. 21.15 *Loading DNA samples into the wells of an agarose gel.*

3 Gel Electrophoresis

Gel electrophoresis is used to separate the DNA fragments of different sizes produced in the PCR. A gel is prepared using a substance called **agarose**. Agarose is a **polysaccharide** derived from seaweed. Molten agarose is poured into a mould and allowed to set. DNA samples are loaded into small wells at one end of the gel (Fig. 21.15). The gel is then placed in a gel electrophoresis tank containing a buffer solution.

DNA is negatively charged so when an electric field is applied it moves away from the negative electrode and towards the positive electrode (Fig. 21.16). Ions in the buffer solution provide charged particles that allow electricity to flow through the gel.

The agarose gel is porous so it allows the fragments of DNA to move through it. However, larger molecules move more slowly than smaller molecules, so the fragments are separated based on their size. The DNA fragments are stained with a fluorescent dye so that they can be visualised. The pattern of bands produced from a DNA sample can then be used as a DNA profile.

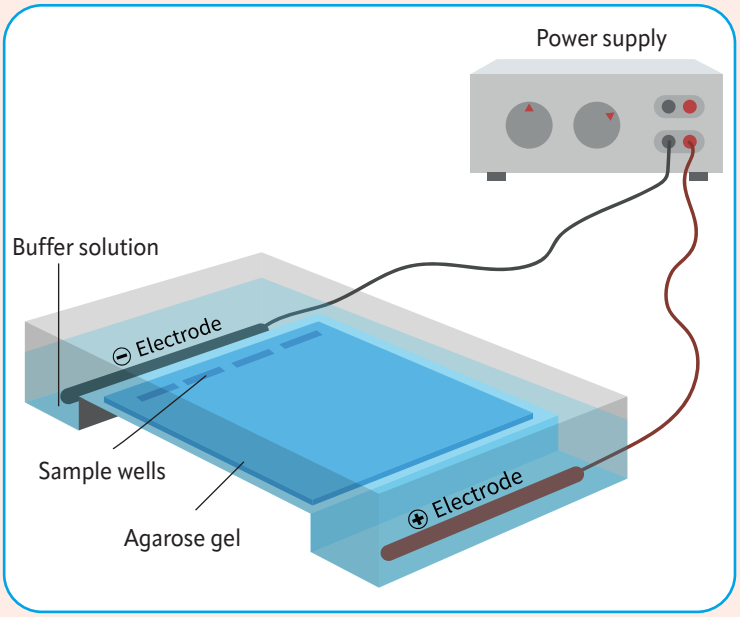


Fig. 21.16 *The agarose gel is placed in a gel electrophoresis tank surrounded by buffer solution and an electric field is applied.*

Link 21.6

Scan this code to watch an animation that explains agarose gel electrophoresis.

Go to page 213 in the **Research and Investigations Book** for an activity on gel electrophoresis.

DNA Sequencing

DNA sequencing is a process in which the order of bases in DNA is determined. It can be used to sequence a single gene or part of a gene, larger regions of a chromosome, or the entire genome of an organism. Like PCR, DNA sequencing technology is based on the process of DNA replication.

Definition: DNA sequencing is a process in which the order of bases in a DNA molecule is determined.

Terminator DNA sequencing was invented in 1977 by Fred Sanger, a UK biochemist. He was awarded a Nobel Prize in 1980.

The Components of DNA Sequencing

- **Target DNA**: the double-stranded DNA molecule to be sequenced, often first amplified by PCR.
- **Primer**: a short single-stranded DNA molecule, normally 20–40 nucleotides long.
- **DNA polymerase**: an enzyme that will synthesise DNA fragments; in the sequencing process, a range of fragments are synthesised, each one nucleotide longer than the next.
- **DNA nucleotides**: a supply of each of the four nucleotides (A, T, G, C).
- **Terminator nucleotides**: four modified terminator nucleotides (A, T, G, C). Each of the four is labelled with one of four different coloured fluorescent dyes. Once incorporated into a DNA strand, they prevent further elongation.
- **Sequencing gel**: an acrylamide gel is used, rather than agarose, because it is capable of separating DNA fragments that differ in size by just one nucleotide. Agarose gel electrophoresis is not suitable for distinguishing between such small differences in size.
- **Gel electrophoresis tank**: long thin gels held in buffer with electrodes at each end of the gel.

The Process of DNA Sequencing

Four DNA sequencing reaction mixes are made in separate tubes. Each contains the target DNA molecule, DNA polymerase, a primer and regular nucleotides. Terminator A nucleotides are added to the first tube, terminator T to the second tube, terminator G to the third and terminator C to the fourth tube.

Template Preparation

The four tubes are heated to a **high temperature**. This breaks the hydrogen bonds between the strands of the double-stranded target DNA molecule, separating them. Only **one** of the strands acts as the **template** strand.

Primer Binding

The reaction mixes are cooled down to allow the **DNA primer** molecule to bind to the template DNA. The primer sequence is complementary to a sequence at the end of the template strand. This is the starting point for the DNA polymerase to begin DNA synthesis.

DNA Synthesis and Termination

DNA polymerase synthesises a complementary strand using regular nucleotides, but there are also some **terminator nucleotides** in the reaction mix. When a terminator nucleotide is added by DNA polymerase, strand **elongation stops** at that point. In this way, a collection of DNA fragments is synthesised.

The reaction tube containing terminator A nucleotides will contain a collection of fragments that all end in A. Similarly, the T tube will contain DNA fragments that all stopped at T. The G tube will only have DNA fragments that end in G, and the C tube fragments all stopped at C.

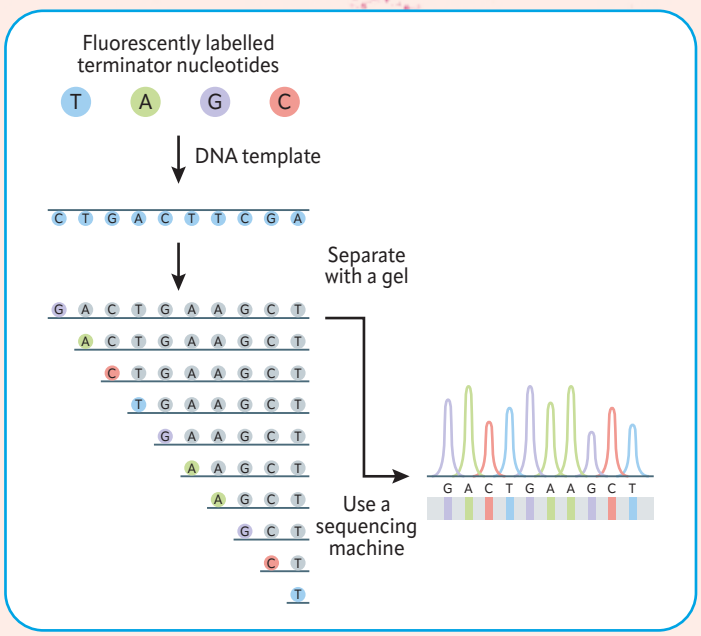


Fig. 21.17 *The DNA sequencing process results in fragments that differ by one base in length. The final nucleotide in each case is fluorescently tagged. The colour depends on whether the base is A, T, G or C.*

Fragment Separation

The collection of fragments is then separated in an acrylamide gel based on their size. Shorter fragments move through the gel more quickly, while longer fragments are slower.

Link 21.7

Scan this code to watch an animation on DNA sequencing.

Link 21.8

Scan this code to watch an animation on DNA sequencing.

Reading the Sequence

As the fragments move through the gel, a laser scanner detects the colour of the fluorescent tag on each fragment. Each fragment that passes through is one nucleotide longer, so the scanner records the order of the fluorescent terminator nucleotides as they pass through. The colour depends on whether the base is A, T, G or C (Fig. 21.17).

Go to page 220 in the **Research and Investigations Book** for an activity on DNA sequencing.

Analysing Genetic Data

The order of bases in a DNA molecule can be determined using DNA sequencing. The process was developed in the 1970s and 1980s. Once the technique became established a project was launched to sequence the entire human genome. The Human Genome Project was carried out between 1990 and 2003 and was a collaborative international effort.

Extend Your Knowledge

Scan this code to learn more about the Human Genome Project.

As the project progressed, so too did advancements in technology. New computer analysis tools were needed to interpret the information contained within the sequencing data. A new type of science called **bioinformatics** emerged, which used information storage and analysis tools to identify patterns in biological data. One of the pioneers of bioinformatics in the 1980s was an Irish scientist, UCD's Professor Des Higgins whose 1988 research paper set the standard for DNA sequence alignment software. It is now one of the top 10 most highly cited scientific research publications of all time.

Around 2–40 billion gigabytes of genomic data is generated every year. The technologies used enable scientists to discover how particular DNA sequences might be linked to human health and disease.

Genetic Data Access

As the Human Genome Project progressed, all of the DNA sequences produced were shared publicly. There is now a large international database of publicly available sequences that includes sequencing data from many organisms, not just humans. This freely available data means that greater progress can be made in scientific research as scientists all over the world have access to a huge store of genetic data.

Go to page 221 in the **Research and Investigations Book** for an activity on using a genome database to search for alleles responsible for genetic diseases.

Genetic Data Use

Agriculture

Use of genetic data in agriculture can help to identify sequences associated with desirable traits in crops and livestock. This information can be used to make decisions about breeding, which can lead to more productive crops and healthier livestock.

Health

Access to genetic data can help to identify DNA sequences that are associated with particular diseases or conditions (see DNA testing).

Industry

Genetic data can be used in the food industry to identify the animal and plant species present in products and to trace ingredients such as meat to a specific source. This enables greater **food security** and can protect against fraudulent claims. For example, in 2013 the Food Safety Authority of Ireland used DNA testing to determine the source of meat found in a number of products, such as ready meals and frozen beef burgers. Out of 27 beef burger products tested, 10 were found to contain horse meat.

Reproduction

Genetic data can be used in reproduction to predict the likelihood of parents passing on a particular disease or condition to their child. It can also be used in conjunction with *in vitro* fertilisation to determine if an embryo carries the gene for a particular condition (see DNA testing).

Forensics

As we saw above, DNA profiles can be used to compare data from one individual to another. Large numbers of DNA profiles are stored in databases, allowing comparisons to be done electronically. For example, the FBI in the United States uses the Combined DNA Index System (CODIS) to compare DNA profiles. CODIS contains approximately 20 million DNA profiles.

DNA Testing

DNA testing is a general term that includes the preparation of a DNA profile and the testing of genes or chromosomes for the presence of **mutations**. Earlier in this chapter we saw how DNA testing can be used in paternity tests (i.e. identifying family relationships) and forensics.

Genetic screening is a form of DNA testing used to identify if a person has a gene mutation associated with a particular disease using available genetic data. While many diseases result from a combination of environmental and genetic factors, some are associated with a single gene mutation. For example, **cystic fibrosis** is most commonly caused by a mutation in a single gene.

Another example is the BRCA gene mutations that are associated with an increased risk of **breast cancer**. Individuals from families with a history of breast cancer, especially cases occurring before the age of 50, can choose to be tested for the gene mutation. A positive test for the mutation allows individuals to make choices that may prevent them from developing the disease. For example, they might have more regular breast screening and/or choose to have surgery or take medicine that could reduce their risk of developing cancer.

Genetic screening can also give parents information about the likelihood of their children having a disorder. Parents from families with a history of a particular condition, who do not have the condition themselves, may be carriers of a gene mutation if the disorder is **recessive**. By screening for the presence of the gene mutation they can make informed decisions before becoming pregnant.

DNA testing can be used alongside ***in vitro* fertilisation** (IVF). **Pre-implantation genetic testing (PGT)** can be carried out on fertilised embryos to check for the presence of mutations or for chromosomal abnormalities. Only embryos without the detected mutations are transferred into the uterus, increasing the chance of a successful pregnancy.

Go to page 217 in the **Research and Investigations Book** for a research task on genomic testing.

Stem Cells

Definition: Stem cells are unspecialised cells that have the potential to become different types of cells.

Stem cells are unspecialised animal cells that have the potential to make more stem cells and to become other types of cells. There are several types of stem cell:

- **Embryonic stem cells** – these are cells that have the potential to become any type of cell in the body. They are obtained from fertilised embryos when they are around 3–5 days old.
- **Perinatal stem cells** – these are found in the umbilical cord and amniotic fluid during pregnancy.
- **Adult stem cells** – these are cells found in small numbers in most adult tissues and have the potential to become a few different types of cells. For example, stem cells in the bone marrow have the potential to become any of the different types of blood cells (Fig. 21.18).
- **Reprogrammed adult stem cells** – these are adult stem cells that have been modified in a laboratory to have properties similar to embryonic stem cells.

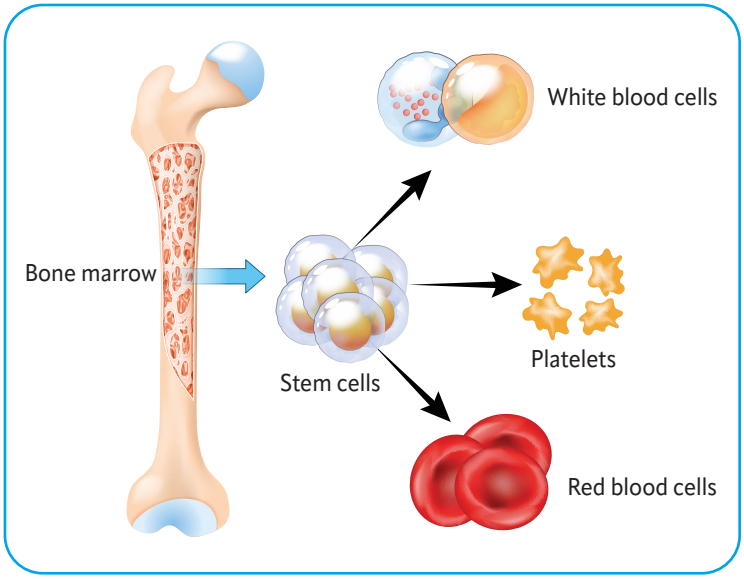


Fig. 21.18 *Blood stem cells in the bone marrow have the potential to become any type of blood cell.*

Stem cells can be studied to understand how the body makes different types of tissue and how it replaces damaged or unhealthy cells. Researchers can use stem cells to test new therapies for particular diseases. The use of embryonic stem cells is controversial as it relies on using fertilised embryos. The development of reprogrammed adult stem cells avoids the use of embryos in stem cell research.

Stem Cell Therapy

Stem cell therapy uses stem cells to repair diseased or injured tissue. Transplants of bone stem cells have already been used successfully. The most common use is in the treatment of leukaemia, a cancer that affects white blood cells. Chemotherapy is used to kill the harmful cancerous cells and they are then replaced with healthy bone stem cells from a donor.

Stem Cells and Cloning

The use of stem cells has the potential to replace organ transplants. While organ transplants save many lives, they are dependent on a donor organ becoming available. In addition, transplanted organs are recognised by the body as foreign, so the **immune system** attacks them. Patients who receive a transplanted organ must take medicine to suppress their immune response, but this can leave them vulnerable to infections.

If stem cells can be stimulated to differentiate into particular tissue types, they could be used to repair damaged or diseased tissue so that organ transplants would not be necessary. If the stem

cells are derived from the individual needing treatment it reduces the risk of the tissue being rejected.

One method that could be used to produce such stem cells is the same as that used to clone animals. The **haploid** nucleus is removed from an egg cell and replaced with a nucleus from a **diploid** body cell of the individual to be cloned. The egg is then stimulated to start dividing, forming an embryo (Fig. 21.19).

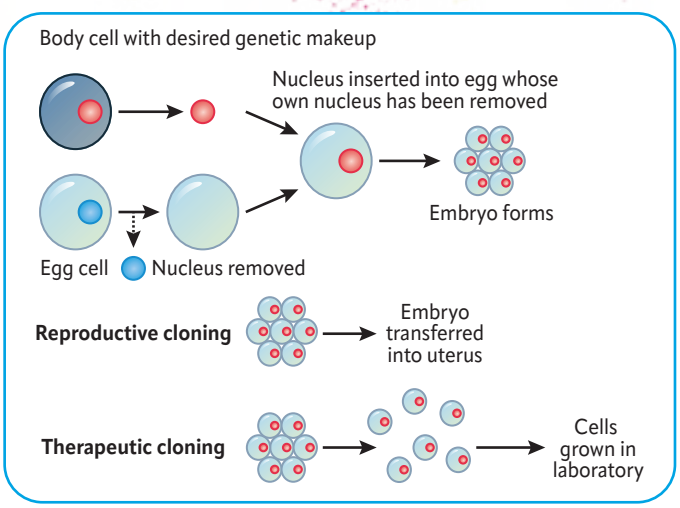


Fig. 21.19 *Reproductive and therapeutic cloning both involve inserting a nucleus from the individual to be cloned into an egg cell that has had its nucleus removed. In reproductive cloning, the resulting embryo is transferred to the uterus of an animal and pregnancy progresses. In therapeutic cloning, the cells are grown in a laboratory and can be used to produce different tissue types.*

Reproductive Cloning

When cloning an animal, the embryo is transferred to a **uterus** and the pregnancy progresses (Fig. 21.19). This is known as **reproductive cloning**.

Reproductive cloning can be used to make more individuals with particular traits instead of relying on breeding. It is particularly useful if a genetically modified animal has been created, as it ensures the animals produced have the modified traits. The first mammal produced by cloning was Dolly the sheep, who was born in 1996 (Fig. 21.20).

Fig. 21.20 *Dolly the sheep was the first mammal to be cloned. The work on animal cloning that led to Dolly being created was carried out at the Roslin Institute in Scotland by Professor Ian Wilmut, shown here with Dolly.*

Therapeutic cloning

To prepare stem cells, the embryo is not transferred to a uterus, but instead the cells are grown in the laboratory (Fig. 21.19). This is known as **therapeutic cloning**. These cells have the potential to become any tissue so could be used to replace damaged tissue. They have the same genetic make-up as the individual who provided the nucleus. This means they are less likely to induce an immune response when introduced into the person's body.

While the process has potential, it does have some drawbacks. For example, if the egg used is from a donor, its **mitochondrial** DNA will differ from that of the patient so there may still be an immune response to transplanted tissue. There are also ethical issues around creating embryos that will then be destroyed to harvest stem cells. In addition, it is seen by some as opening the door to human cloning.

Therapeutic cloning of human cells has not been successfully carried out yet, but researchers continue to study the process.

Advantages and Disadvantages of Genetic Technologies

Advancements in genetic technologies offer opportunities for progress in many aspects of life, in particular in medicine, agriculture and industry. However, as with any new technology, they can also have negative impacts. Some of the advantages and disadvantages are outlined below.

Advantages of Genetic Technologies

- Earlier diagnosis of some diseases, for example, some forms of breast cancer.
- Potential to repair gene mutations that cause hereditary diseases or predispose people to cancer.
- Genetically modified white blood cells offer a promising way to treat cancers.
- Can make crops disease resistant and able to grow in difficult conditions, which will help as the effects of climate change become more widespread.
- Can make livestock healthier and more productive, reducing herd sizes.
- Can help to stop the spread of disease by modifying genes of insect hosts.

Disadvantages of Genetic Technologies

- Sharing of genetic data could affect people's eligibility for insurance or could lead to discrimination.
- Gene editing in early human embryos could lead to the possibility of 'designer babies', raising ethical questions.
- Modification of an embryo's DNA would not only affect the child's DNA but any descendants of that child.
- Genetic engineering could be used to make more dangerous infectious diseases for biological warfare.

Go to page 217 in the **Research and Investigations Book** for an activity on the ethical issues arising from advancements in genetic technologies.

Chapter Summary

Biotechnology and Genetic Engineering

Genetic Engineering

- **Biotechnology** is the use of organisms or biological systems to make products or carry out processes that are useful.
- **Genetic engineering** is the manipulation of the DNA of an organism.
- A **restriction enzyme** is an enzyme that cuts the DNA molecule at a specific base sequence.
- **DNA ligase** is an enzyme that forms bonds between the sugar and the phosphate group of adjacent nucleotides in a DNA molecule.
- A **plasmid** is a small circular DNA molecule found mainly in prokaryotic cells.
- Genetic engineering involves the following four steps: DNA isolation, cutting, ligation, transformation and expression.
  - **Isolation** of DNA from cells.
  - **Cutting** of DNA with restriction enzymes. Both the gene of interest and the plasmid DNA are cut with the same enzyme.
  - **Ligation** of the gene of interest and the plasmid DNA. A **recombinant plasmid** is a plasmid containing a fragment of foreign DNA.
  - **Transformation** is a process where bacteria take up DNA from their environment.
  - **Gene expression** is the final stage of the process, where the information in the gene is used to produce a protein.
- Genetic engineering has applications in:
  - **Medicine** – for example, the production of human insulin by bacterial cells.
  - **Agriculture** – for example, to produce pest-resistant crops.
  - **Industry** – for example, for the production of enzymes.
- A **genetically modified organism (GMO)** is an organism that has been altered using genetic engineering techniques.

The Polymerase Chain Reaction

HL The **polymerase chain reaction (PCR)** is a process used to make many copies of a particular section of DNA. It involves the following components:

- **DNA template** – a sample of the DNA containing the region to be copied.
- **DNA nucleotides** – a supply of each of the four nucleotides (A, T, G and C).
- **DNA polymerase** – the enzyme that adds the nucleotides onto a strand of DNA. The DNA polymerase used in PCR (*Taq* DNA polymerase) is heat resistant.
- **DNA primers** –short single-stranded DNA molecules complementary to each end of the region to be copied.
- **PCR buffer** – a solution that maintains the pH.

HL **Denaturation** involves separation of the two strands of the target DNA molecule. Each strand acts as a template.

HL **Annealing** is where the primers bind (attach) to the complementary sequences on the template strands. One primer binds at one end of one strand of the target DNA; the second primer binds to the opposite strand at the other end of the target sequence.

HL **Extension** is when the *Taq* DNA polymerase enzyme adds free nucleotides to their complementary bases on the template strands at the end of the primer sequences.

HL Applications of PCR include use in DNA profiling and detection of infectious agents, such as the SARS-CoV-2 virus.

DNA Profiling

- **DNA profiling** is the process of making a unique pattern of bands from a person's DNA that can be distinguished from the pattern of another person.
- A person's **DNA profile** is prepared by separating fragments of their DNA **based on size**.
- Its most common applications are in determining **family relationships** and in **forensics**.

HL The variable regions most commonly used in DNA profiling are called **short tandem repeats** (STRs).

HL The steps involved in preparing a DNA profile are extraction of DNA from cells, PCR of variable regions and separation of DNA fragments based on size using agarose gel electrophoresis.

DNA Sequencing

HL DNA **sequencing** is a process in which the order of bases in a DNA molecule is determined.

HL A collection of DNA fragments is generated, each of which is one nucleotide shorter than the next, and these fragments are separated using gel electrophoresis. Fragments that all end in a particular nucleotide are all the same colour, and there is a different colour for each of the four nucleotides. The colours are detected by a laser scanner as they pass through the gel, one after another.

- The order of bases in a DNA molecule can be determined using **DNA sequencing**.
- Large international databases provide free access to **genetic data**, allowing scientists to use it for their research.
- Genetic data is used in:
  - **Agriculture** – for example, to identify genes for favourable traits in livestock and crops.
  - **Health** – for example, in diagnosis of genetic conditions.
  - **Industry** – for example, to identify animal and plant species present in products.
  - **Reproduction** – for example, to predict the likelihood of a couple passing on a particular disease to their children.
  - **Forensics** – for example, in DNA profiling.
- **DNA testing** applications include determining family relationships, forensics and genetic screening.
- **Genetic screening** uses available genetic data to identify the presence of mutations.

Stem Cells

- **Stem cells** are unspecialised cells that have the potential to become different types of cells.
- There are several types of stem cells:

- **Embryonic stem cells** – obtained from fertilised embryos.
- **Perinatal stem cells** – found in the umbilical cord and amniotic fluid during pregnancy.
- **Adult stem cells** – found in small numbers in most adult tissues.
- **Reprogrammed adult stem cells** – adult stem cells that have been modified in a laboratory to have properties similar to embryonic stem cells.

- **Stem cell therapy** uses stem cells to repair diseased or injured tissue.
- **Reproductive cloning** can be used to clone whole animals using embryos prepared in the laboratory.
- **Therapeutic cloning** uses cloned cells to prepare tissue for medical treatments. This has the potential to replace the use of organ transplants.

Advantages and Disadvantages of Genetic Technologies

Advantages include:

- Earlier **diagnosis** of some diseases.
- Potential to **repair** gene mutations.
- Production of **disease-resistant crops**.
- Production of healthier, more productive **livestock**.
- Prevention of spread of some **infectious diseases**.

Disadvantages include:

- Sharing of genetic data could lead to **discrimination**.
- Possibility of **designer babies**.
- **Future generations** could be affected by changes made to embryos.
- Possibility of use in **biological warfare**.

Questions

1. **(a)** What is meant by the term *biotechnology*?

(b) State two traditional examples of biotechnology.

(c) What is meant by *genetic engineering*?

2. **(a)** What is a restriction enzyme?

(b) Restriction enzymes occur naturally in bacterial cells. What is their function?

(c) Describe how restriction enzymes recognise where to cut DNA.

(d) What is meant by the term *sticky ends*?

(e) How does the presence of sticky ends help the process of genetic engineering?

(f) Name one restriction enzyme and the base sequence it recognises.

3. **(a)** What is the function of the enzyme DNA ligase?

(b) State two processes in the cell that involve DNA ligase.

(c) What is a plasmid?

(d) State three features often found in plasmids modified for use in genetic engineering and describe how they aid the process of genetic engineering.

4. **(a)** Outline the four steps involved in genetic engineering.

(b) What is meant by the term *recombinant plasmid*?

(c) Name the vessel used to grow large numbers of bacterial cells for expression of the gene of interest.

(d) Give an example of an application of genetic engineering in (i) medicine, (ii) agriculture and (iii) industry.

(e) What is meant by the term *genetically modified organism*?

HL **5.** **(a)** What is the polymerase chain reaction (PCR)?

(b) List the components required for PCR.

(c) Explain why a heat-resistant DNA polymerase is used in PCR.

(d) Outline the three stages involved in PCR.

(e) Describe two applications of PCR.

(f) Outline the advantages of using PCR in genetic engineering, instead of cutting the gene of interest out of a DNA sample.

6. **(a)** What is meant by *DNA profiling*?

(b) State two uses of DNA profiling.

HL **(c)** Describe what short tandem repeats are and how they are used in DNA profiling.

(d) State the three steps involved in preparing a DNA profile.

(e) What property of DNA causes it to move through the electric field in gel electrophoresis?

(f) What causes the DNA fragments to separate based on their size?

7. **(a)** What is meant by *DNA sequencing*?

(b) What are terminator nucleotides and how are they useful in DNA sequencing?

(c) Describe how gel electrophoresis is used in DNA sequencing.

(d) Describe how DNA sequencing data can be used in (i) agriculture and (ii) industry.

8. **(a)** Name two types of DNA testing.

(b) What is meant by *genetic screening*?

(c) Give two examples of the use of genetic screening.

(d) Describe how DNA testing can be used with *in vitro* fertilisation to increase the chances of a successful pregnancy.

9. **(a)** What is meant by the term *stem cells*?

(b) Describe the four different types of stem cells.

(c) Outline how stem cells can be used to treat disease.

(d) Describe how the use of stem cells could replace organ transplants.

(e) Outline the process used to prepare a cloned embryo.

(f) Distinguish between *reproductive* and *therapeutic cloning* and give an application of each.

10. **(a)** Outline three advantages of genetic technologies.

(b) Outline three disadvantages of genetic technologies.

(c) Choose one advantage that, in your opinion, justifies the use of genetic technologies and give a reason for your answer.

(d) Choose one disadvantage of the use of genetic technologies that you think poses the greatest risk and give a reason for your answer.