TOPIC 6: IMMUNITY, INFECTION AND FORENSICS

For the Edexcel Biology A Level (SNAB)

TOPICS COVERED

- Forensics: PCR, Electrophoresis and Interpretation
- Determining Time of Death including Body Temperature, Rigor Mortis, Decomposition and Forensic Entomology
- Non-Specific Immunity - Inflammation and Phagocytosis
- Specific Immunity - B and T cells
- *Bacteria Case Study: Tuberculosis*
- Antibiotics and Building Resistance
- Viruses - *Case Study: HIV and AIDS*
- mRNA splicing
- Preventing Entry of Pathogens
- Developing Immunity and Vaccinations

Forensics

Key Terminology

Term	Definition
Introns	Intragenic Regions – non-coding DNA
Exons	Expressed Regions – coding DNA
Short Tandem Repeats	Short DNA sequences that are repeated many times within introns
Polymerase Chain Reaction	A reaction that amplifies DNA that is found at a crime scene
DNA Primers	Short DNA sequences complementary to the DNA adjacent to the STR
Autolysis	The body's enzymes break down cells

DNA Profiling

Conventional methods for identification include fingerprinting and using dental records. Increasingly, DNA profiling may be used to identify either a body or a suspect. DNA profiling relies in the concept of a unique genome, particularly the number of STR repeats within introns. An STR can be repeated from five to a few hundred times. The same STRs occur on both chromosomes of a homologous pair, but the number of repeats on each can vary. The immense variation in the repeats present means that combinations of STRs are virtually unique. DNA profiling visualises a profile, before comparing to a reference for identification.

1. Obtaining DNA

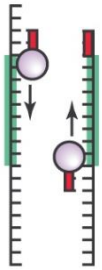
DNA can be obtained from biological tissue sample. The sample is broken down in a buffer solution containing salt and detergents, which disrupt membranes within the cell. Suspended particles are then separated from cell debris by filtering. Protease enzymes break down remaining proteins, and addition of cold ethanol causes precipitation of DNA out of the sample.

2. Creating Fragments

The DNA sample is treated with restriction endonucleases, which cut DNA at specific base sequences either side of an STR sequence.

3. Amplifying DNA – Polymerase Chain Reaction

In order for minute samples from a crime scene, such as skin or bodily fluids, to be used for identification, DNA must be copied using the polymerase chain reaction.



a) A DNA sample is placed in a reaction tube in a PCR thermal cycler with DNA polymerase, DNA primers with fluorescent markers and nucleotides
b) At 95°C, the DNA strands separate as the hydrogen bonds between strands break
c) At 55°C, primers attach to the start of the STR sequence
d) At 72°C, DNA polymerases bind to the primers and nucleotides are added, extending DNA from the primer, thereby replicating the STR sequence and adjacent DNA
e) The cycle repeats, producing 2^n repeats of the original sample, where n is the number of cycles (~25-30)

4. Separating Fragments

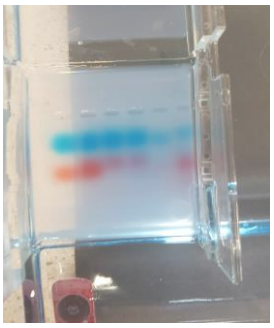
The DNA fragments produced are then separated by gel electrophoresis.

a) An agarose gel is poured in an electrophoresis tank. Wells are made in the gel using a comb
b) Samples of DNA have a blue dye added, before being loaded into wells using a micropipette
c) The gel is submerged in a buffer and electrodes are connected, producing a potential difference. The negatively charged DNA mopves towards the anode. Smaller fragments with fewer STRs travel faster
d) A reference sample with fragments of known lengths may be added to produce a DNA ladder

To visualise the fragments if the dye is insufficient:

e) DNA is denatured to single strands and transferred to from the fragile gel to a nylon filter membrane during Southern blotting
f) The membrane is placed in a bag with a radioactive DNA probe – these single stranded probes bind to the fragments which are complementary to it. When an X-ray film is used to cover the nylon, after development the positions of the probe and therefore the STR sequences are visible

5. Interpreting DNA Profiles



STR sequences can be measured based on base pair length after electrophoresis. The banding patterns can be used to compare a crime scene sample to a suspect's sample.

The fragments of DNA produced by the PCR may be analysed by electrophoresis in a different way if the primers have a fluorescent tag attached. As the tags and the STRs pass through a laser, the time taken for movement of STR through the gel can be identified. Several STR loci can be analysed simultaneously if tags give off colours of different wavelengths. A graphical DNA profile will indicate peaks along the x-axis corresponding to the size of a DNA fragment. If a single peak, or band on an electrogram, is observed, then the same number of repeats is inherited from each parent. If two peaks are observed, then different repeats are seen on each chromosome.

The STRs are inherited in the same way as alleles, with offspring inheriting a sequence randomly from each parent. As a result, DNA profiling can be used in identification, paternity tests, and observing variation.

DNA profiling is widely used in legal proceedings to produce almost unique results which almost certainly indicate guilt of a suspect. Since DNA profiles only observe STRs, there is a chance that the profiles are actually not unique; this becomes a significant issue if suspects are closely related.

Determining Time of Death

Body Temperature

After death, a decrease in core body temperature immediately begins, allowing estimates for time of death up to roughly 24 hours. Core temperature is measured via the rectum or abdomen. Environmental conditions must be notes as these can affect cooling. The cooling of a body follows a sigmoidal curve, eventually plateauing at ambient temperature. Other factors such as body size, position, air movement and immersion in water also affect cooling.

A Henssge nomogram can be used to identify time of death by linking ambient temperature, mass of the corpse and its core body temperature

Rigor Mortis

After death, muscles totally relax then stiffen. This stiffening is rigor mortis. Immediately after death, muscle cells are starved of oxygen. Respiration in cells becomes anaerobic, producing lactic acid. The pH of the cell falls, inhibiting enzymes and glycolysis. The ATP needed for muscle contraction is no longer produced and bonds between actin and myosin become fixed, also fixing muscles and joints.

Rigor mortis passes after 6-9 hours as muscle tissue starts to break down as lysozymes release enzymes.

Decomposition

- Autolysis: enzymes from the body, from lysozymes and the digestive tract, break down cells
- Bacterial Invasion: bacteria from the gut and respiratory system rapidly invade tissues after death, releasing digestive enzymes
- Putrefaction (37-72 hours): greenish discolouration of the lower abdomen due to sulfur combining with haemoglobin. This spreads around the body, turning reddish-green then purple-black. Gases and liquid blisters appear on the skin
- Bloating (7 days): gases from bacterial action (H_2S, CH_4, NH_3, H_2 etc) form in the intestine and cause smelling and bloating
- Dehydration: fluid from putrefaction drains away, and soft tissue shrinks and decay rate drops

Forensic Entomology

Forensic entomology is the use of insects that inhabit decomposing remains to aid legal investigations by identifying the species' present on a corpse and figuring out the stage in its lifecycle it is in.

Insects are attracted to humid openings or wounds. Identification of a maggot's stage of development with reference to the life cycle of a fly can give an estimate of age. The time of egg-laying can be found by the date of pupation subtract the time for egg development. The time laying gives an estimate of time for date of death as blowflies lay eggs within one day of death. When factors such as temperature which affect lifecycles are considered, this provides a fairly accurate estimate for date of death.

In corpse succession, as one group feeds on a decomposing body, the conditions change in such a way that it becomes attractive to another wave – this provides a predictable sequence which can be used to determine time of death. The season can influence the pioneer species. Insects can also help determine if a body has been moved – if insects found in the woods are on a body discovered indoors, this suggests movement.

Decomposers

Insects and bacteria from the gut often invade a corpse immediately after death. However, bacteria and fungi from the surroundings also contribute to the decay of a body. A corpse is a great energy source for decomposers due to the vast array of organic material available. CO_2 is released into the atmosphere by respiring decomposers, recycling carbon back into a form used in photosynthesis. Decomposition is a major process within the carbon cycle.

The Immune System

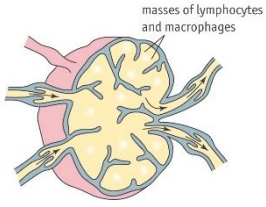
Key Terminology

Term	Definition
Non-Specific Responses	The body's general response to any invading pathogen
Specific Responses	The reactions of B cells and T cells, types of lymphocyte, to particular antigens form the specific immune response
Inflammatory Response	The characteristic reddening and swelling around a wound
Phagocytosis	Engulfing and destruction of bacteria and cell debris by macrophages
Lymph Nodes	Part of the lymphatic system which filters the lymph passes through the node, any pathogens present are destroyed by lymphocytes
Interferon	A substance produced in non-specific immunity against viruses
B Cells	B lymphocytes are produced in bone marrow and bind to antigens with a complementary shape
T Helper Cells	Cells produced bone marrow but mature in the thymus, which stimulate clonal selection of B cells and production of T killer cells
T Killer Cells	Bind to complementary antigens and cause infected cells to undergo lysis
Antigen Presenting Cell	Macrophages displaying non-self antigens to activate T-helper cells
Cytokines	Chemicals released by activated T helper cells which stimulate division and differentiation of B and T cells

The immune system aims to prevent infection by invading pathogens. Non-specific responses help destroy any pathogen, whilst specific immunity controls a specific pathogen.

Non-Specific Responses

- **Lysozyme** is an enzyme found in tears, saliva and nasal secretions that kills bacteria by breaking down cell walls
- **Inflammation** at sites of injury after a blood clot seals the wound
    a. Damaged white blood cells and mast cells release histamine
    b. Histamine causes neighbouring arterioles to dilate, increasing blood flow in capillaries at infection site, and also increasing capillary permeability
    c. Plasma fluid, white blood cells and antibodies leak into the tissue, causing oedema (swelling)
    d. Infecting microbes can be destroyed by white blood cells
- **Phagocytosis** occurs when white blood cells – first neutrophils, then macrophages – engulf foreign matter
    a. White blood cell engulfs bacterium or cell debris and encloses this ingested material in a vacuole
    b. Lysosomes containing digestive enzymes fuse with the vacuole, causing destruction of foreign matter
    c. After a few days, the site if full of dead cells, forming pus, which is often reabsorbed by tissue

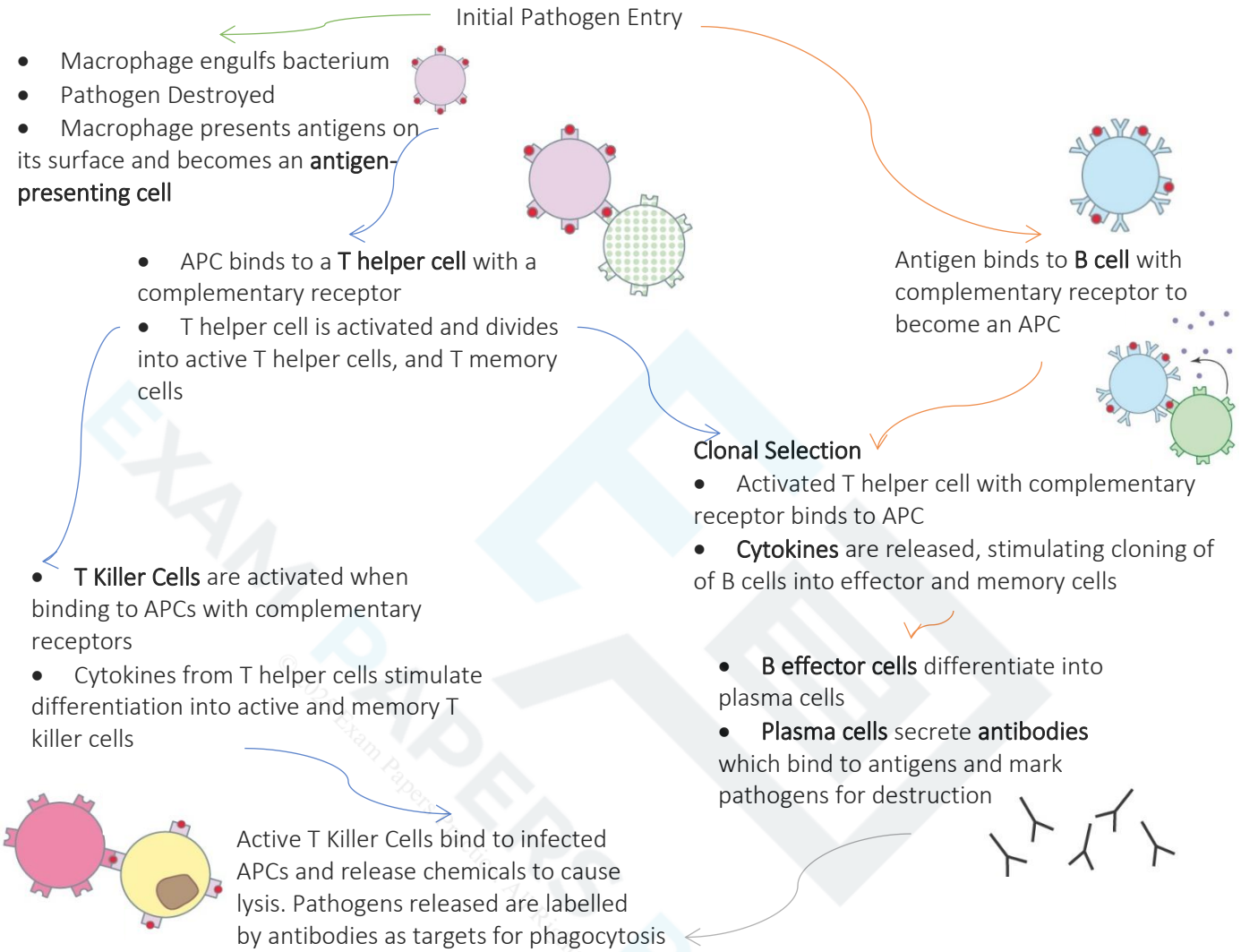


- **Lymph Nodes** contain a mass of lymphocytes and macrophages, to ensure that any live bacteria which enter the blood and the lymph do not spread through the body. If this fails, septic shock results
- **Interferon** provides deference against viruses; infected cells release interferon which diffuses to surrounding cells where microbe multiplication is prevented, by inhibiting microbial protein synthesis

Specific Immunity

Primary Immune Response

Specific immunity is dependent on lymphocytes, of which there are two types: B and T cells. Both types respond to non-self antigens.



Secondary Immune Response

If infected by the same pathogen again, the immune system responds much faster, as memory cells are involved. B memory cells are used to produce antibodies, at a faster rate and in greater quantities – the rapid response means that the person is often unaware of any symptoms. The person is now immune to the disease.

Bacteria: Tuberculosis and Antibiotics

Key Terminology

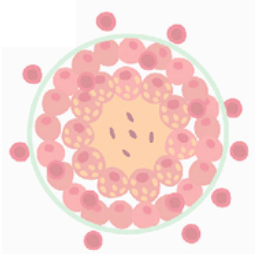
Term	Definition
Pathogen	A microorganism that causes disease
Gram-Positive Bacteria	Bacteria with cell walls that are thickened with additional polysaccharides and proteins
Tubercules	Anaerobic masses of tissue containing dead bacteria and macrophages
Bactericidal Antibiotics	Antibiotics that destroy bacteria
Bacteriostatic Antibiotics	Antibiotics that prevent multiplication of bacteria
Vertical Evolution	Genes are passed on between generations
Conjugation	Direct cell-to-cell contact between pili of bacteria
Horizontal Evolution	Genes are passed from one bacteria to another, which may be of a different species, through plasmid transfer

Bacteria are prokaryotes which lack a nucleus and membrane-bound organelles. Bacteria divide asexually by binary fission. The structure of bacteria was studied in Topic 3.

TB is a droplet disease, carried in mucus and saliva released into the air when an infected person sneezes or talks. *Mycobacterium tuberculosis* is a tough bacterium which causes TB, a highly contagious disease, which affects the respiratory system. It is estimated that 1.5 million people die from TB every year, with 6 million new cases every year. Infection begins when the bacteria is inhaled and lodged in the lungs, where they start to multiply. Symptoms of TB include coughing up of blood, shortness of breath, weight loss or fever.

An individual with TB experiences fever because, as part of the inflammatory response, neutrophils and macrophages release fever-causing substances which affect the hypothalamus, increasing the set core body temperature to 40.5°C in order to enhance immune function, and reduce pathogen growth rate.

Primary Infection



- TB is attacked by T cells and macrophages after the bacteria is inhaled
- As TB has a tough waxy cell wall, it resists breakdown after phagocytosis, surviving within macrophages
- In order to prevent spread of infection, the immune cells form a ring around the infected site. This mass is known as a granuloma. In TB, this anerobic mass, containing dead bacteria and macrophages – is a tubercule
- After 3-8 weeks, the infection is controlled and the infected lung heals. Live but inactive bacteria remain within the tubercules, with numbers controlled by the immune system

Active Tuberculosis

- Active Tuberculosis occurs if the immune system cannot control the disease when it first arrives, or if the immune system weakens due to age or AIDS
- Bacteria multiply rapidly and destroy lung tissue by cavity creation

TB can also infect bones, lymph nodes and the CNS, often following initial pulmonary infection. In glandular TB the main symptom is enlarged lymph glands.

TB Diagnosis

TB is diagnosed by analysis of symptoms, a skin test for TB antigens, a blood test for T cells specific to TB antigens, identification of bacteria by staining, and chest X-rays.

Treating TB: Antibiotics

Antibiotics are chemicals produced by microorganisms with the ability to inhibit growth of or destroy bacteria. They have no effect on eukaryotic cells. Antibiotics are produced by microorganisms to help them compete in the environment. The first drug which was used to treat TB was streptomycin.

There are two types of antibiotic: **bactericidal** antibiotics destroy bacteria, whilst **bacteriostatic** antibiotics prevent multiplication of bacteria, allowing the host's immune system to then destroy the pathogens. There are several methods used by antibiotics to interfere with bacteria:

- Inhibition of cell wall synthesis, leading to lysis of weak walls
- Disruption of the cell membrane, causing permeability changes and resulting in lysis
- Inhibition of nucleic acid synthesis and replication, preventing cell division
- Inhibition of protein synthesis or inhibition of bacterial enzymes

Disease has been a part of human life since our evolution, as pathogens and hosts coexist. Selection pressures exerted by pathogens has resulted in human mutations leading to disease resistance. At the same time, pathogens are continuously evolving new methods to overcome the immune system. They may also evolve resistance to antibiotic treatment as some may have mutations which result in an enzyme which breaks down the antibiotic. Bacterial populations in particular can evolve very quickly, due to a short lifecycle, and vast population sizes and genetic diversity. This leads to an evolutionary race.

Vertical evolution of bacteria is when advantageous alleles are passed along generations. However, **horizontal evolution** can also occur, where resistance genes are passed between bacteria by conjugation. The plasmid carrying the gene for

antibiotic resistance splits into its strand, and once strand transfers between conjugating bacteria. Each bacterium then replicates the strand to create a complete plasmid, giving both bacteria resistance.

There is now widespread TB resistance to streptomycin and other bacteria. Resistant bacteria reproduce faster with reduced competition. Since there are now fewer new antibiotics being produced, bacteria appear to be catching up. Some bacteria are multiple-resistant, and are common in hospitals. MRSA, for example, can cause dangerous infections.

In response to increasing spread of healthcare associated infections, hospitals are attempting to improve infection control by having hand wash stations, and preventing certain clothing for health workers. Antibiotics should only used strictly when necessary, and patients should complete their treatment even after they feel better, in order to destroy all remaining bacteria.

Viruses: HIV and AIDS

Key Terminology

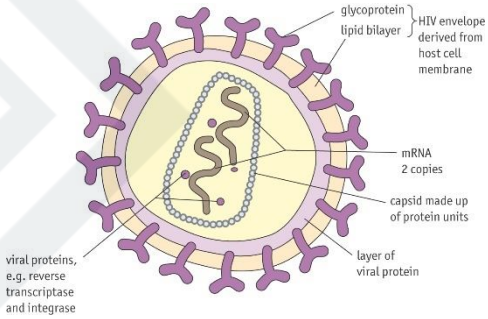
Term	Definition
Viruses	Small organic disease-causing particles made of nucleic acid and a protein coat
mRNA Splicing	The removal of introns and the joining of exons from pre-mRNA

Virus Structure

Viruses consist of a strand of nucleic acid enclosed in a protein coat. Viral DNA can be single or double stranded. Viruses vary greatly in size, shape and complexity, and must infect and enter host organism cells in order to make more viruses. The hijacking of the host cell causes disease. After reproduction, the new virus particles burst out of the cell during lysis, resulting in damage to the surrounding tissue. Viral envelopes contain antigens.

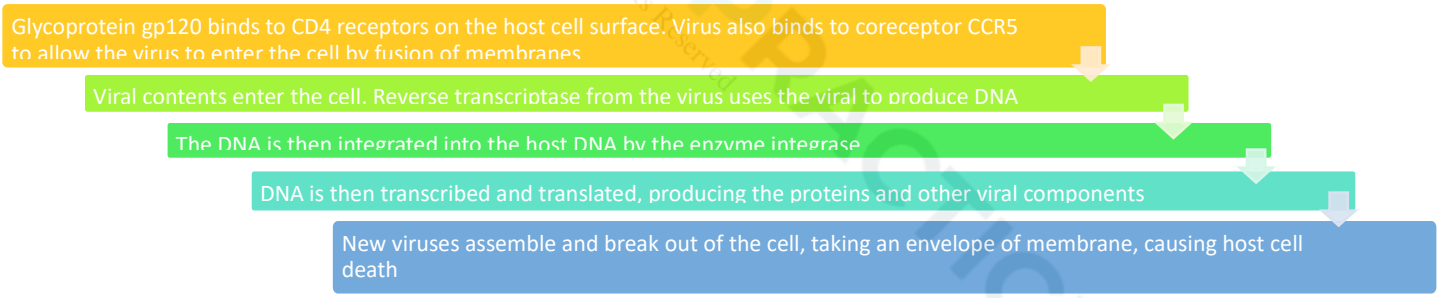
HIV Structure and Transmission

AIDS, acquired immune deficiency syndrome, is caused by infection with HIV, human immunodeficiency virus. HIV is a complex virus, consisting of RNA surrounded by an icosahedral protein capsid enclosed in a layer of viral protein, through which glycoproteins project. There is also an envelope that is derived from the host cell membrane when the virus emerges.



HIV can only be passed on through bodily fluids, such as blood, vaginal secretions and semen. Infection can occur through unprotected sex, blood transfer through cuts, sharing needles for drugs, and maternal transmission.

HIV Infection



HIV viruses infect T helper cells and therefore infection has great impact on the immune system, as macrophages, B cells and T killer cells are not activated and therefore do not function properly. The deficiency of the immune system means the patient will eventually develop AIDS. However, first the disease progresses through several stages:

Phase	Impacts
Acute	• Fever, sweating, headaches, sore throat, swollen lymph nodes are common, although there may be no symptoms • HIV antibodies appear in the blood after 3-12 weeks • Rapid replication of the virus, loss of T helper cells. After a few weeks, infected T helper cells are recognised by T killer cells, which begin to destroy them, reducing but not eliminating the virus population
Chronic	• Prolonged, latent phase where the virus reproduces rapidly with numbers controlled by the immune system • There may be no symptoms, although there is an increasing tendency to suffer other infections which last for longer • Dormant diseases, such as TB, may reactivate • Often lasts many years, up to 20 with drug treatment, but can last just a few years in LEDCs

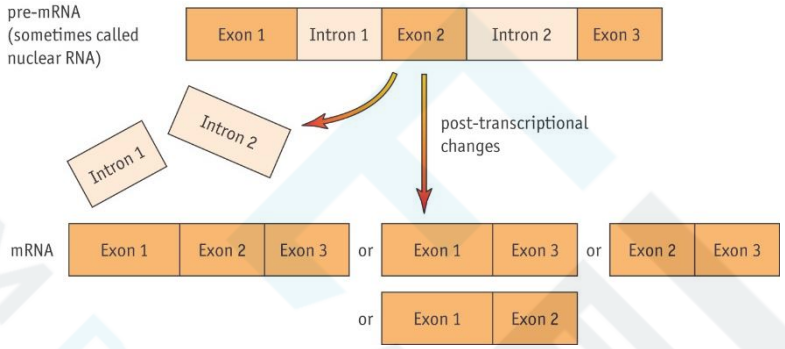
Disease *(AIDS)*	• Increased viral load in circulation and declining T helper cells indicates the onset of AIDS • Increased vulnerability to other diseases, and opportunistic infections (pneumonia, TB etc) can become rapidly fatal • Significant weight loss, risk of dementia and certain tumours rises

Treating AIDS

HIV cannot be removed as the virus is hidden inside T helper cells. However, there are drugs, known as antiretrovirals, which reduce production of new viruses. A cocktail of antiretrovirals is used to avoid resistance to anti-HIV drugs. Drugs involved include: reverse transcriptase inhibitors, preventing viral RNA from making DNA; protease inhibitors, preventing catalysis of cutting large proteins into short chains for viral construction; integrase inhibitors; fusion inhibitors. Side effects may include nausea, fatigue, damage to peripheral nerves, and liver and kidney damage.

mRNA Splicing

Between transcription and translation, mRNA is edited, with non-coding regions – introns – being removed. The remaining regions, which are to be translated and expressed, are exons. The exons are then joined in different ways, meaning several different, but related, proteins are produced from a single mRNA strand that is spliced in different ways. This alternative splicing is strictly controlled so the correct protein is produced.



Preventing Infection

Key Terminology

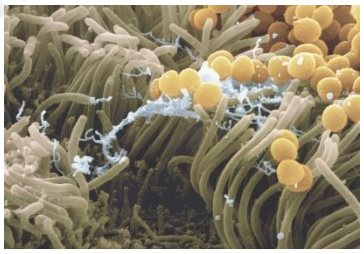
Term	Definition
Skin Flora	Microbes that live on the skin surface
Lysozyme	An enzyme that breaks down bacterial cell walls, causing the cell to burst
Immunity	Destruction of pathogens before onset of any symptoms
Passive Immunity	Short-term immunity, where an individual produces no antibodies themselves
Active Immunity	Longer-term immunity, where an individual produces memory cells for a pathogen
Artificial Immunity	Immunity that requires medical treatment
Natural Immunity	Immunity that occurs naturally, by immune response, or between mother and child

Preventing Entry of Pathogens

Skin

The skin's keratin layer is an effective, continuous barrier to microorganisms. Entry can occur through any wounds, but blood clotting seals wounds to prevent infection. Skin flora live on the skin surface. These microorganisms are harmless and well adapted to the skin surface environment, and prevent colonisation by other bacteria. Other bacteria are not as well suited to the conditions created by sweat and urea.

Mucous Membranes



The mucous membranes lining the airway and gut provide easy routes of entry to the body, as the moist surface is favourable for bacterial growth. Entry is limited by mucus, secreted by goblet cells in the trachea and bronchi, and cilia, which sweep away microbes and other particles trapped by the mucus. Secretions in the mouth, eye and nose contain lysozyme, an enzyme which breaks down bacterial cell walls, causing the cell to burst.

Digestive System

Gastric juices produced by the gastric glands in the stomach walls contain hydrochloric acid, giving a pH under 2.0, killing most bacteria that enter the stomach in food, but also giving the optimum pH for the digestive enzyme pepsin.

Gut Flora

Hundreds of bacterial species are found in the intestines, benefiting from the warm and moist conditions whilst aiding digestion and competitively excluding pathogenic bacteria in this mutualistic relationship. The gut flora also secrete chemicals which are useful in defence against pathogens.

Becoming Immune

People can become immune to a specific disease in various ways, detailed in the tables below

Active Artificial Immunity	Immunity that develops following immunisation. Antigens in the vaccine trigger a specific immune response by the body's immune system
Passive Artificial Immunity	Immunity that develops when a person is given ready-made antibodies, providing immediate but short-lived protection in emergency situations, as antibodies are quickly broken down
Active Natural Immunity	Immunity that develops following an infection. The specific immune response to the non-self antigens helps destroy the pathogens and produces a supply of antibodies and B and T memory cells that will respond quickly if re-infected with the same pathogen
Passive Natural Immunity	Immunity that develops when antibodies pass from a mother to baby either across the placenta in utero, or via breast milk after birth. This protects the baby for a short time

Vaccinations and Active Artificial Immunity

When being vaccinated for a particular disease, the immune system responds to the vaccine in the same way at it responds to the disease; antibodies and memory cells give long-lasting protection. However, the vaccination does not stop contraction of the pathogen; instead, the immune system rapidly destroys it before onset of any symptoms.

Vaccines must contain antigens found on particular pathogens. This can be achieved by injecting one of:

- Attenuated viruses - weakened and harmless
- Dead bacteria
- Toxin that is altered to a harmless form
- Antigen-bearing fragment of a pathogen

Vaccinations may require boosters to ensure lasting immunity. Vaccinations protect entire communities by herd immunity, if over 95% have been immunised, as there is little risk of coming into contact with infected individuals.

There is currently no vaccine for AIDS, but vaccines for TB do exist and are used for high-risk groups.

Vaccinations may cause mild soreness, fever or feelings of nausea, with very occasional long-term impacts. The balance of risk and benefit is very important to consider.