Topic 6: Immunity, Infection and Forensics

SPECIFICATION

6.1 Understand how to determine the time of death of a mammal by examining the extent of decomposition, stage of succession, forensic entomology, body temperature and degree of muscle contraction.

6.2 Know the role of microorganisms in the decomposition of organic matter and the recycling of carbon.

6.3 Know how DNA profiling is used for identification and determining genetic relationships between organisms (plants and animals).

6.4 Know how DNA can be amplified using the polymerase chain reaction (PCR).

6.5 Be able to compare the structure of bacteria and viruses.

6.6 Understand how Mycobacterium tuberculosis (TB) and Human Immunodeficiency Virus (HIV) infect human cells, causing a sequence of symptoms that may result in death.

6.7 Understand the non-specific responses of the body to infection, including inflammation, lysozyme action, interferon, and phagocytosis.

6.8 Understand the roles of antigens and antibodies in the body’s immune response including the involvement of plasma cells, macrophages and antigen-presenting cells.

6.9 Understand the differences between the roles of B cells (B memory and B effector cells) and T cells (T helper, T killer and T memory cells) in the body’s immune response.

6.10 Understand how one gene can give rise to more than one protein through post-transcriptional changes to messenger RNA (mRNA).

6.11 i) Know the major routes pathogens may take when entering the body.
  ii) Understand the role of barriers in protecting the body from infection, including skin, stomach acid, and gut and skin flora.

6.12 Understand how individuals may develop immunity (natural, artificial, active, passive).
SPECIFICATION

- 6.13 Understand how the theory of an ‘evolutionary race’ between pathogens and their hosts is supported by the evasion mechanisms shown by pathogens.
- 6.14 Understand the difference between bacteriostatic and bactericidal antibiotics.
- 6.15 Know how and understand the contributory causes of hospital acquired infections that have led to codes of practice regarding antibiotic prescription and hospital practice that relate to infection prevention and control.
It is often important for police and the forensic experts to establish a body's time of death (TOD). The information regarding TOD can be achieved by looking at several factors together. There are total of five factors that are important for this:

a. Body temperature:
   - The human body produces heat from metabolic reactions like respiration.
   - After death, the body temperature starts falling due to the metabolism stopping.
   - The human body cools at a rate of around 1.5°C to 2.0°C per hour.
   - Different conditions affect the cooling rate such as clothing, air temperature, and body weight.

b. Muscle contraction
   - Rigor mortis (stiffening of the muscle) sets in around 4-6 hours after death.
   - This occurs due to lack of oxygen supply to the muscles and lactic acid production due to anaerobic metabolism.
   - Due to increased lactic acid production, pH of the muscle becomes less.
   - The bonds between the actin and myosin become fixed due to absence of ATP, leading to rigor mortis.

c. Forensic entomology
   - After death, different microorganisms start colonising the body.
   - Identification of the type insects present in and on the body helps to establish TOD.
   - Identifying the stages of life cycle the insects are in (e.g. presence of blowfly eggs or larvae) also helps to estimate time of death.

d. Extent of decomposition
   - Forensic scientists can use the extent of decomposition by microorganisms to establish the time of death. For e.g., cells and tissue are broken down by the body's own microorganisms in first few hours to a few days after TOD.
   - Temperature and oxygen availability also affects the tissue decomposition by microorganisms.

e. Stages of succession
   - The changing types of organisms found on a dead body also helps to identify TOD.
   - Immediately after death, the bacteria invades the body.
   - As the body decomposes, flies start colonising the body and then beetles enter.
   - Beetles decompose dry tissues and complete the decomposition of the body leaving behind only the skeleton.
   - This is affected by the location of the body such as above ground or in water etc.
· Microorganisms such as bacteria and fungi play an important role in decomposing organic matter.

· After an organism, either plant or animal, dies microorganisms start growing on them.

· They secrete enzymes that change the dead organic matter to smaller molecules.

· These are eventually recycled back to the atmosphere as methane (CH4) and CO2.
● DNA profiling can be used to determine the genetic correlation between two organisms, either animal or plants.

● It is the genetic fingerprint of an organism’s DNA.

● This technique is based on the principle that everyone has different DNA, so the DNA profiling will be different for everyone.

● In closely related organisms, the DNA profiling will be similar.

● A greater number of DNA bands on the electrophoresis gel will be matched for closely related species.
PCR or polymerase chain reaction amplifies DNA and make millions of copies of specific regions in a very small amount of time. The procedure of PCR is as follows:

- Setting up the reaction mixture containing primer, the DNA sample to be amplified, free nucleotides, and the DNA polymerase enzyme.
- Sample is heated to 95°C and cooled to 55°C to facilitate the binding of the DNA primers with the DNA strand.
- Again, the temperature is set to 72°C for DNA polymerase to work.
- DNA polymerase makes strands of new DNA by complement strand synthesis.
- Each cycle doubles the amount of DNA present in the sample, and this process repeats.

The polymerase chain reaction
Structure of Bacteria:
- Single-celled.
- Cell wall is made up of glycoproteins.
- Also have an outer membrane called a plasma membrane.
- Are very small in micrometers.
- Have cytoplasm, cell organelles including ribosomes.
- DNA is freely floating inside the cytoplasm, don’t reside inside the nucleus.
- Bacterial cells have hair-like structures known as pili (small) and flagellum (long).

Virus structure:
- Are smaller than the bacteria.
- Plasma membrane, cytoplasm, or ribosome is absent in virus.
- The nucleic acid is either DNA or RNA.
- The outer membrane is called capsid.
- Some viruses also have an outer layer called an envelop that is formed from the cell membrane of a previous host cells.

Source: Slideshare.net (bacteria), wikipedia.org (virus)
Acquired Immune Deficiency Syndrome
- HIV enters the body through the body fluids of the infected person.
- Entering the host cell, it attaches itself on the host’s T cells.
- The capsid is released inside the host cells.
- Inside the cytoplasm of the host cells, the genetic material (RNA) is released.
- Using this RNA as a template, reverse transcriptase produces complement DNA.
- Viral proteins start to be produced inside the host T cells.
- These viral proteins then forms new viruses, which come out from the T cells and start infecting other T cells.
- As the infection progresses, the helper T cells decrease in the body eventually causing a failing immune system.

Tuberculosis
- Tuberculosis is caused by a bacteria called Mycobacterium Tuberculosis.
- Infection is caused by inhalation of the bacteria into the lung.
- Inside the lung, the bacteria is taken up by the white blood cells or phagocytes.
- Inside the phagocyte cells, the bacteria replicates and lives in a tubercle.
- In individuals with weak immune systems, the bacteria gets reactivated and overcomes the immune system causing the tuberculosis infection.
Inflammation
- After a foreign antigen enters the body, the site of infection becomes red, swollen and painful. This reaction is called inflammation.
- After the pathogen enters the body, different immune modulatory molecules are produced.
- This triggers the widening and increased permeability of the blood vessels.
- Increased blood flow brings immune cells to the site of infection.
- Immune cells enter the infected tissues and kill the pathogens.

Interferons
- They are antiviral proteins that prevent virus infection in healthy cells.
- The mechanism of action of interferon involves inhibiting viral replication.
- Activates cells of the specific immune responses against the virus.
- Promotes inflammation to the site of infection.

Phagocytosis and lysozyme
- Phagocytosis is carried out by phagocytes.
- Phagocytes are also called antigen presenting cells (APCs).
- They are the first line of defence against the pathogen.
- The mechanism of action of phagocytes are as follows:
  - Recognises the pathogen and engulfs it, forming a phagocytic vacuole.
  - Lysozyme containing various digestive enzymes then fuses with the phagocytic vacuole.
  - Enzymes present in the lysozyme then breaks the pathogen into smaller antigens.
  - These small antigens then stick on the surface of the phagocytic cells and presented to the T or B cells.
Activation of T cells by macrophages

- T cells have receptors present on cell surfaces.
- These receptors binds with the complementary antigen present on the surface of macrophages (a type of antigen presenting cells).
- This binding activates the T cells.
- Activated T cells carry out different functions such as:
  - T helper cells secrete substances that activate the B cells.
  - T killer cells kill the pathogen infected cells.
  - T memory cells are responsible for secondary immune response.

B cell activation and plasma cells

- B cell is a type of white blood cell and its activation is done by T helper cells.
- They are covered with antibodies.
- Upon recognition of an antigen, they form antigen-antibody complexes by binding with a complementary antigen.
- Every B cell binds with the specific antigens.
- Plasma cells are clones of B cells.
- They secrete antibodies specific for a particular antigen.
- Antibodies clear infections by:
  a. Agglutination reactions
  b. Neutralising the toxins secreted by the pathogens and
  c. Preventing the pathogen from binding with the uninfected host cells.
• T helper cells help activate the B cells by secreting different substances.
• T killer cells kill the pathogen infected cells by attaching itself on it.
• T memory cells are produced after being exposed to any antigens. They remain in the circulation after the pathogen is destroyed. If the same pathogen enters the body again, these memory T cells get activated and cause a secondary immune response.
• Effector B cells are responsible for producing antibody-antigen complexes and help clear infection by agglutination reactions, neutralising toxins, and preventing the pathogen from infecting a host cell.
• Memory B cells are produced similarly to memory T cell and produce plasma cells.
Introns are noncoding regions of the DNA present on a gene. They are separated by exons that codes for particular amino acids. During transcription, introns and exons both get copied. However, during post transcriptional modifications through splicing, the introns get removed. The remaining exons are then joined and this acts as a template during translation of proteins. In this way, one mRNA gives rise to more than one protein by post transcriptional modifications.

Post transcriptional modifications: Splicing of introns

Image source: http://cnx.org/contents/lyDdZqp4@6/Protein-Synthesis
i) There are four major routes through which pathogens can enter:
   - Cut skin
   - Digestive system via contaminated food
   - Through the respiratory system
   - Other mucosal surfaces such as through genitals, nose, or mouth.

ii) a. Skin and skin flora in protecting body from infection:
   - Human skin comprises of three layers -
     Epidermis
     Dermis
     Subcutis (Hypodermis).
   - Epidermis provides a dry, watertight barrier with sloughing dead cells (keratinocytes).
   - Dermis produces sweat and sebum, maintaining acidic pH that prevents most bacteria from growing.
   - Dermis also contains additional molecules that attack invading pathogen by a process known as “phagocytosis” (e.g. neutrophils, macrophages etc).
   - Commensal microorganisms present on the skin (skin flora) secrete fatty acids that inhibit colonisation by other microbes.

b. Stomach acid:
The acidic environment of the stomach kills most of the pathogens that enter through the digestive system.

c. The gut:
The gut or intestine contains harmless microorganisms (called flora) that compete with the pathogen for nutrition and space. This activity restricts the pathogen from infecting the body.
Natural immunity:
When an individual gets exposed to a live pathogen,
Develops a primary immune response and the memory cells remain in the circulation
The immunity acquired by him is natural.
This type of immunity is known as natural immunity.

Artificial immunity:
When immunity occurs because of vaccination then that type of immunity is called artificial immunity.

Active immunity:
When the body’s immune system starts making antibodies after getting exposed by an antigen the immunity is called active immunity. For eg. In vaccination body is exposed to the harmless dose of an antigen and starts producing the antibodies against the antigen.

Passive immunity:
In this type of immunity antibodies made by another organism is given to boost the immune system of another. For eg. In natural passive immunity baby gets its primary immunity through the antibodies it receives from its mother.
The immune system of the Vertebrates has evolved over millions of years. Pathogens also have devised mechanisms to evade the immune system of the host cells.

This struggle between the vertebrate hosts and the pathogen is known as the evolutionary race.

As a result, pathogens have developed some evasion mechanisms.

For example, the Human Immune Deficiency Virus (HIV) kills the host T cells, thereby reducing the number of effective immune cells of the body. It also mutates the genes responsible for producing antigenic proteins by a process known as antigenic variation. This helps the viral proteins to evade recognition by memory cells. So, every time the host cell immune system has to produce a different primary response for a new strain of HIV.

HIV disrupts the antigen presentation by APCs.

Not only HIV, even Mycobacterium tuberculosis also has some evasion mechanisms as follows: Inside the lung’s phagocytic cells, M. tuberculosis prevents the lysozyme from fusing with the phagocytic vacuoles. Thereby, preventing killing of the bacterium by the phagocytosis. This bacterium also prevents the antigen presentation by APCs, thus preventing killing by phagocytosis.
Chemicals that kills or prevents the growth of bacteria are called antibiotics. They are drugs that are used as a treatment for bacterial infection.

There are two types of antibiotics that are used for preventing bacterial infection as follows:

1. Bactericidal: this type of antibiotics kills the bacteria directly. They prevent the enzymes involved in the bacterial cell wall formation. This weakening of the cell wall causes water to move inside the cell leading to cell death.

2. Bacteriostatic: this type of antibiotics slows down the growth of the bacteria either by preventing the protein synthesis or DNA replication or any other cellular metabolism of the bacterium.
Hospital acquired infections (HAI) are infections which are acquired in the hospital and in most of the time are transmitted by the poor hygiene observed by the hospital stuffs and visitors.

The patients have weakened immune system so they are prone to the infection. Certain code of practises if implemented can prevent this HAI. They are as follows:

- Wash hands before and after visiting the patients. This rule is applicable for hospital stuffs as well as visitors.
- Disinfect the hospital beds and surfaces more frequently
- Move the HI patients to certain isolation ward specific for such infections.

Some of the HAI are caused by antibiotic resistant bacteria. This type of infection is difficult to treat as no antibiotic will work on them. For this type of infections also a code of conduct has been generated as such that will prevent bacteria from becoming antibiotic resistant. The rules are as follows;

- Doctors shouldn't prescribe antibiotics for minor bacterial infections or viral infections.
- Prevent doctors from prescribing antibiotics for minor infections and for viral infections.
- Antibiotics shouldn’t be prescribed for preventing infections.
- Prescribe narrow-spectrum antibiotics that are specific for a particular bacterium, if possible.
- Rotate the antibiotic prescribed.
- Patients should adhere the treatment regimen that they have prescribed. They should also complete the full course of the antibiotic prescribed by the doctor so that no infection is left behind.