YISHUN INNOVA JUNIOR COLLEGE

H2

EXTENSION TOPIC A: INFECTIOUS DISEASE

Learning Outcomes:

(a) describe the specific (adaptive) immune system, including active, passive, naturally acquired and artificially acquired immunity, and the non-specific (innate) immune system

(b) outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses

(c) explain the relationship of the molecular structure of antibodies to their functions, using immunoglobulin G, IgG, as an example

(d) explain how somatic recombination, hyper-mutation and class switching result in millions of different antibody molecules

(e) discuss how vaccination can control disease (including the eradication of small pox), limited to vaccination stimulates immunity without causing the disease and vaccination of a high enough proportion of the population can break the disease transmission cycle

(f) discuss the benefits and risks of vaccination

(g) explain how viruses, including influenza virus and HIV, cause diseases in humans through the disruption of host tissue and functions (including HIV and helper T cells, influenza virus and epithelial cells of the respiratory tract)

(h) explain the mode of transmission and infection of bacterial pathogens, using *Mycobacterium tuberculosis* as an example

(i) describe the modes of action of antibiotics, including penicillin, on bacteria

References:

1. Peter Wood, Understanding Immunology, 3rd Edition:
    - Chapters 1 – 10 & 14
2. Campbell & Reece, Biology, 11th Edition:
    - Chapter : Viruses, Pg. 427 – 439 (or corresponding chapter in previous editions)
3. Janeway, *et. al.*, Immunobiology, 6th Edition:
4. Alberts *et. Al.*, Molecular Biology of the Cell, 4th Edition
    - Chapter 24: The Adaptive Immune System, Pg. 1363 – 1420

Note: Some of these textbooks are available in our library. You may wish to borrow them to supplement your reading when necessary.

LECTURE OVERVIEW

1 INTRODUCTION

- **Immunity** is defined as resistance to disease, specifically infectious disease. A disease is termed as **infectious** when it is caused by a pathogen and is able to be transmitted from one host to another.
- The collection of cells, tissues, and molecules that mediate resistance to infections is called the **immune system**. The function of the immune system is to prevent infections and to eradicate established infections. The coordinated reaction of the cells and molecules in the immune system to infectious microbes is the **immune response**.
- **Immunology** refers to the study of the immune system, including its responses to microbial **pathogens** and damaged tissues and its role in disease.

PRACTICES OF SCIENCE (POS)

POS 1: Understanding the Nature of Scientific Knowledge

The origin of immunology is usually attributed to Edward Jenner. He discovered in 1796 that cowpox, or vaccinia, induced protection against human smallpox, an often fatal disease. Jenner called his procedure vaccination, and this term is still used to describe the inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.

1796

Edward Jenner

During the late 19th century, Robert Koch proved that infectious diseases are caused by microorganisms, each one responsible for a particular disease, or pathology. We now recognize four broad categories of disease-causing microorganisms, or pathogens: viruses, bacteria, pathogenic fungi, and parasites.

19th C

Robert Koch

In the 1880s, Louis Pasteur devised a vaccine against cholera in chickens, and developed a rabies vaccine that proved a spectacular success upon its first trial in a boy bitten by a rabid dog.

These practical triumphs led to a search for the mechanism of protection and to the development of the science of immunology.

1880s

Louis Pasteur

In 1890, Emil von Behring and Shibasaburo Kitasato discovered that the serum of vaccinated individuals contained substances—which they called antibodies—that specifically bound to the relevant pathogen.

1890

Emil von Behring & Shibasahuro

1.1 PATHOGENS

- The types of pathogens that can cause disease include many single-celled microorganisms e.g. viruses, bacteria, yeasts and larger multicellular parasites e.g. fungi and parasitic worms.

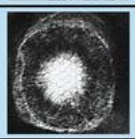
Type of pathogen	Description	Human diseases caused by pathogens of that type
Bacteria *Escherichia coli* 	Single-celled organisms without a nucleus	Strep throat, staph infections, tuberculosis, food poisoning, tetanus, pneumonia, syphilis
Viruses *Herpes simplex* 	Thread-like particles that reproduce by taking over living cells	Common cold, flu, genital herpes, cold sores, measles, AIDS, genital warts, chiken pox, small pox
Fungi *Death cap mushroom* 	Simple organisms, including mushrooms and yeasts, that grow as single cells or thread like filaments	Ringworm, athlete's foot, tinea, candidiasis, histoplasmosis, mushroom poisoning
Protozoa *Giardia lamblia* 	Single-celled organism with a nucleus	Malaria, "traveler's diarrhea" giardiasis, trypanosomiasis ("sleeping sickness")

Fig 1.1.1: Types of pathogens and diseases caused

- Pathogens have many different antigens that can be recognised by cells of the immune system.
  - **Antigens** are substances that **induce an immune response** as they are **recognised by antibodies and cells** of the immune system. Antigens are usually foreign proteins, but can also be carbohydrates, lipids or nucleic acids.
  - For example, a bacterium's antigens can be found on its cell wall and flagellum, while a virus's antigens can be found on its viral glycoprotein.

- Cells of the immune system and antibodies bind to a specific part of the antigen known as the **epitope**. For a protein antigen, the epitope is usually between 8 to 22 amino acids long.

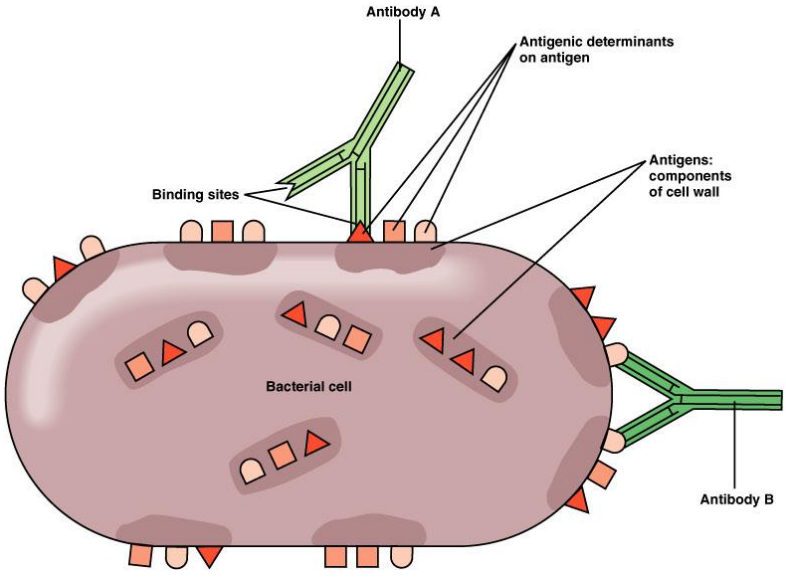


Fig.1.1.2: Antigenic epitopes

1.2 THE IMMUNE SYSTEM

- The immune system functions to remove or neutralise the threats posed to the body by pathogens.

- The organs of the immune system are divided into the primary and secondary lymphoid organs.

  – The **two primary lymphoid organs** (in most mammals) are the **bone marrow** and **thymus**, where naïve lymphocytes develop and maturation occurs – in bone marrow for B cells and thymus for T cells.

  – Mature lymphocytes will then migrate to the **secondary lymphoid organs** and tissue which are the **lymph nodes**, **spleen** and **mucosa surfaces**.

- The **lymphatic vessels** connect most tissues in the body with lymph nodes and eventually the bloodstream. The fluid in the lymphatic vessels is known as **lymph** and is derived from the interstitial fluid surrounding cells in any tissue or organ.

- Lymph contains lymphocytes and tissue-derived dendritic cells and other white blood cells e.g. neutrophils, basophils etc, but not red blood cells. A number of these lymphatic vessels meet at specialized structures known as the lymph nodes.

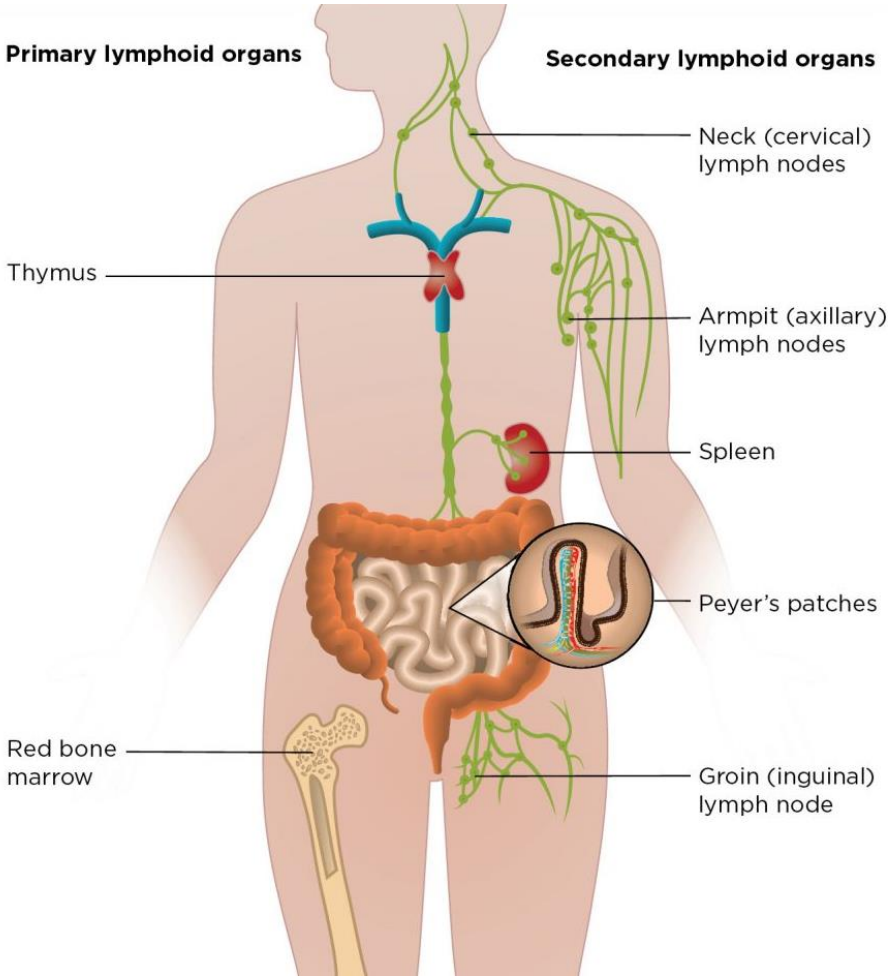


Fig. 1.2.1: Lymphoid organs

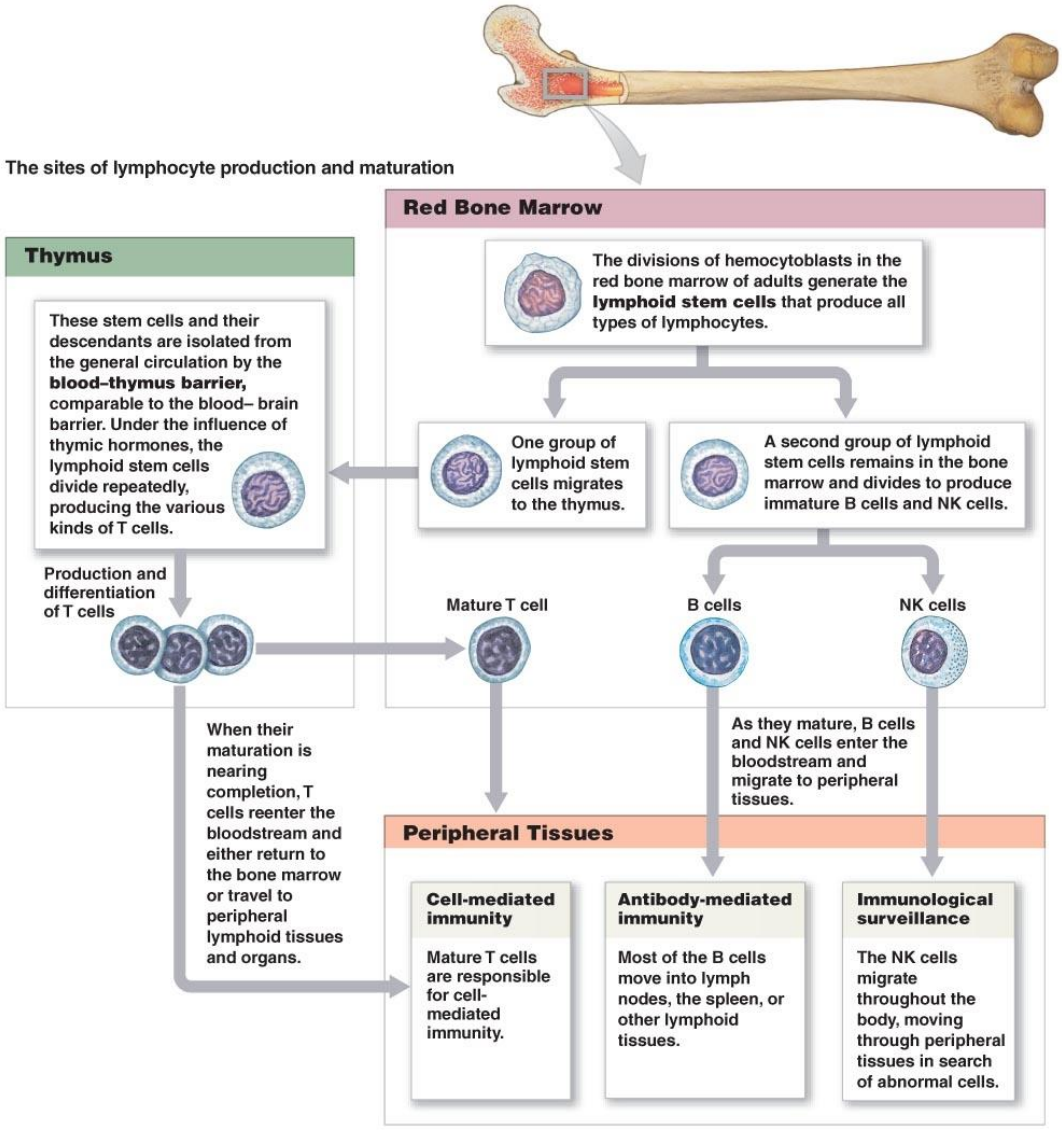
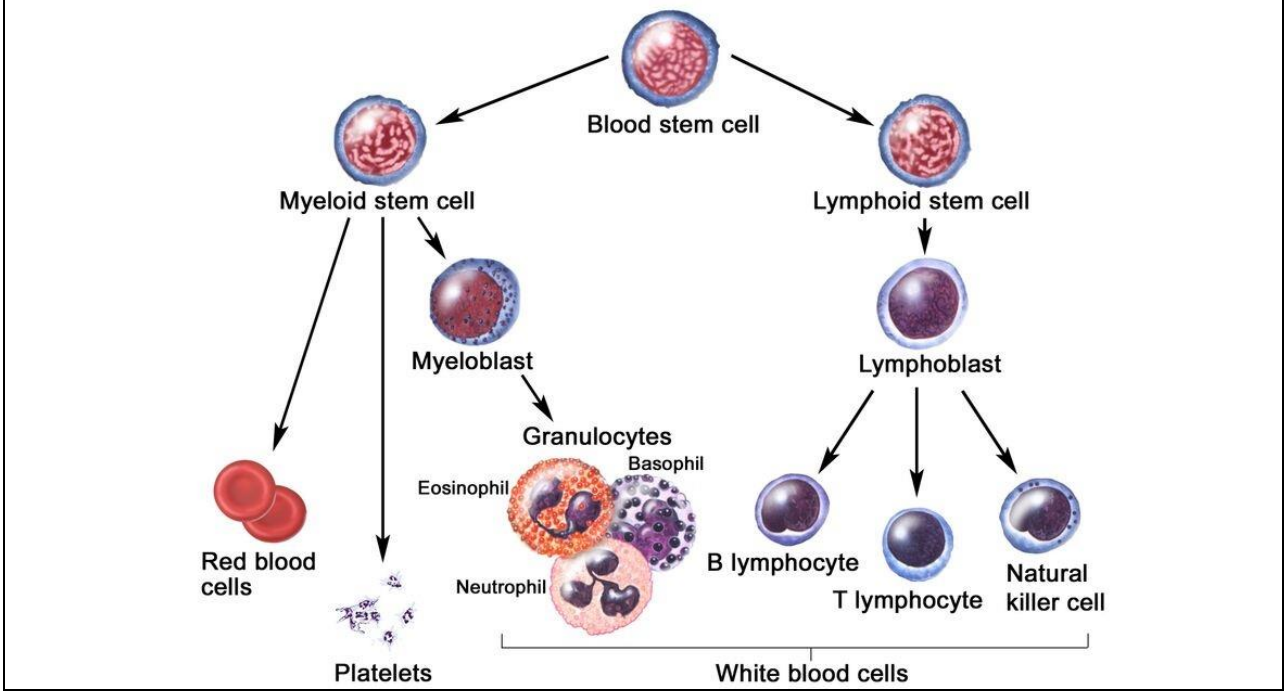


Fig. 1.2.2: Lymphocyte development

RECALL

Differentiation of Haematopoietic Stem Cells



2 IMMUNE RESPONSE

Learning Outcome (a)
Describe the specific (adaptive) and non-specific (innate) immune systems including active and passive, natural and acquired immunity.

- **Innate immunity** involves the immediate response to an infection. It includes a variety of protective measures that are continually functioning, and provides a first line of defence against pathogens. However, it does not have a memory and is **non-specific**.

- **Adaptive immunity** develops more slowly, but provides a **specific** response and long lasting protection against the same pathogen.

  – Adaptive immunity can be induced by exposure to a foreign antigen resulting in the production of antibodies (i.e. **active immunity**), or acquired through the transfer of antibodies from one individual to another (i.e. **passive immunity**).

  – Adaptive immunity can be acquired either through natural means (i.e. **natural immunity**) or artifically (i.e. **artificial immunity**).

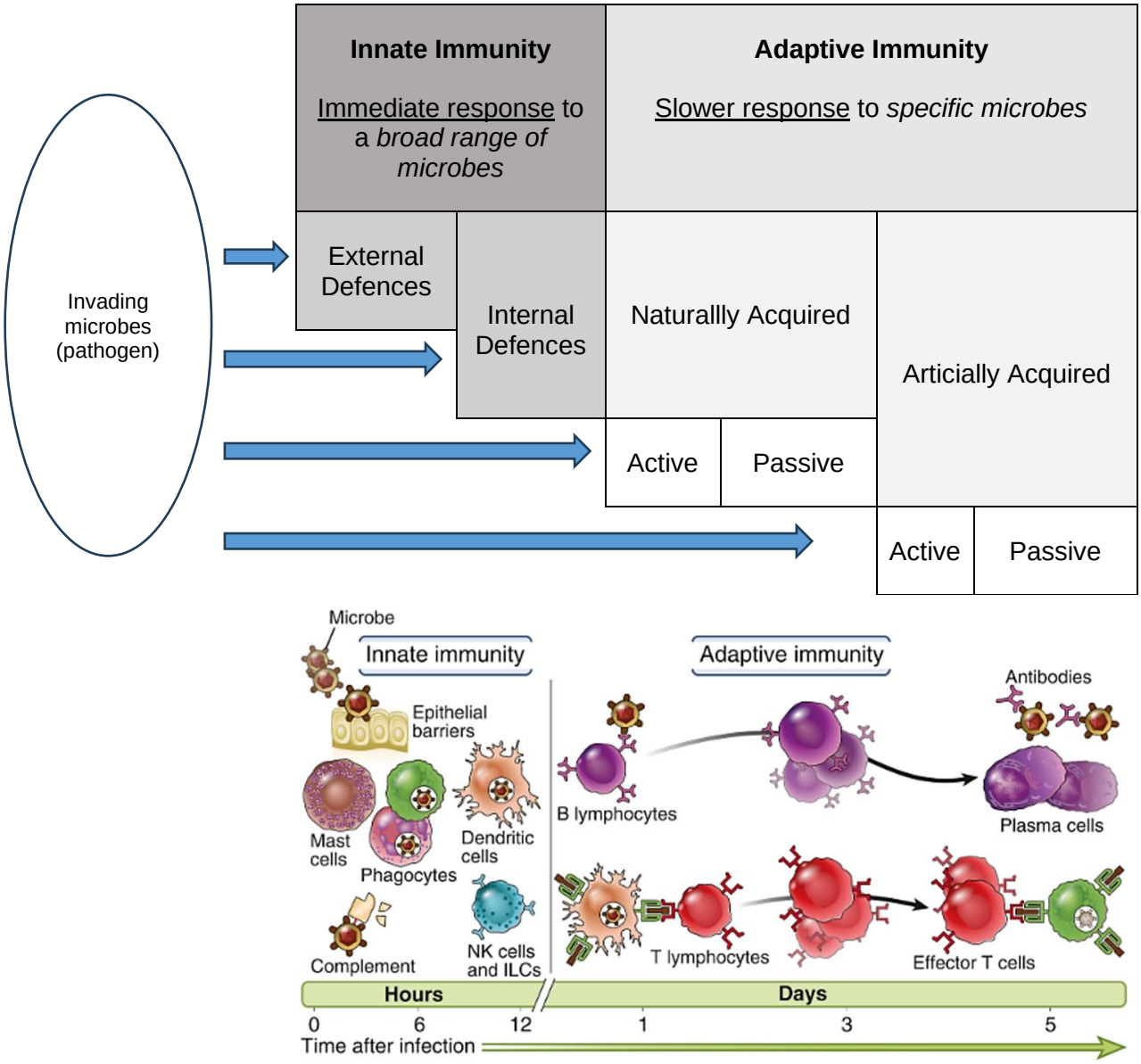


Fig. 2.1: Innate and Adaptive Immunity

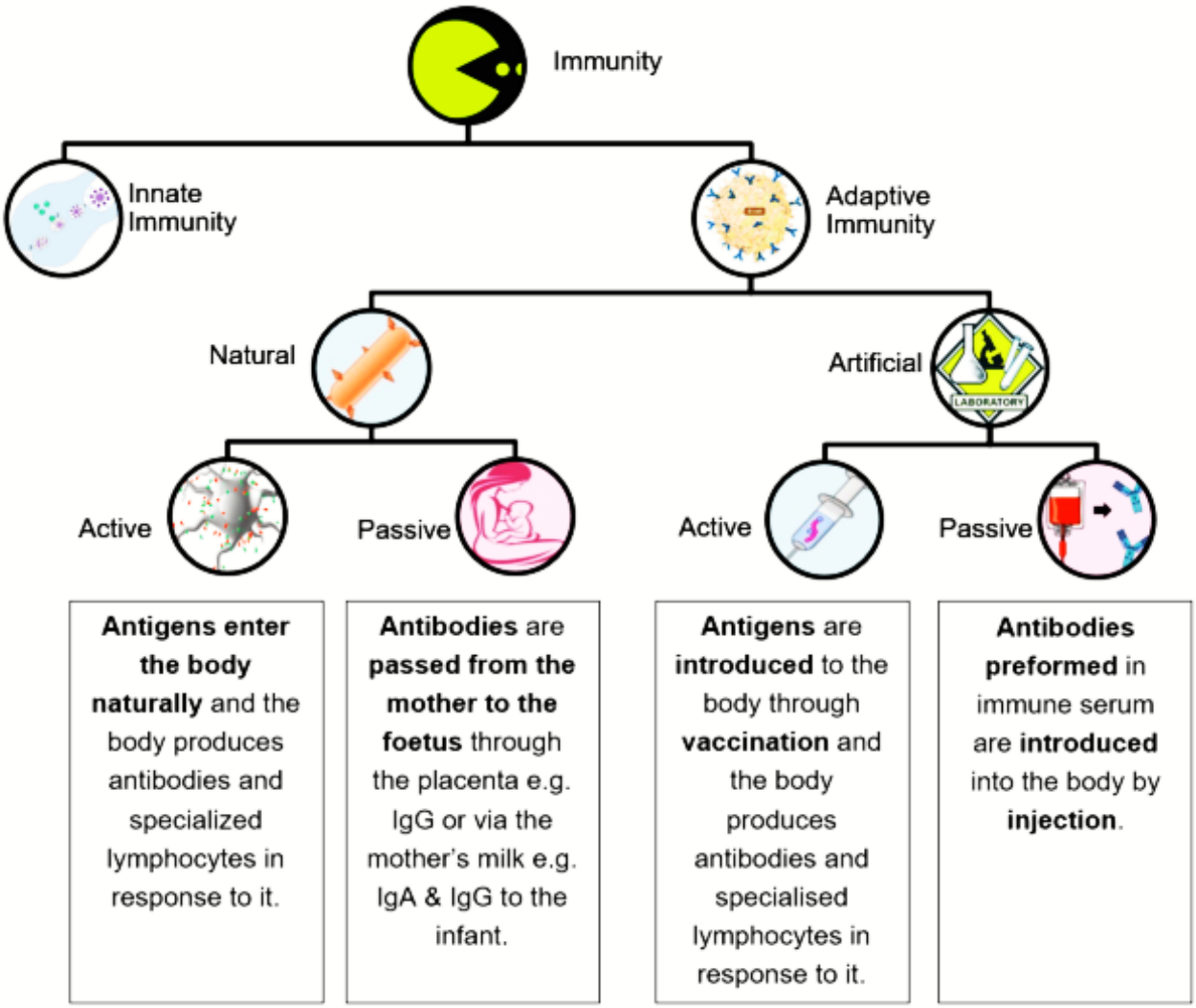


Fig. 2.1: Types of Immunity

Despite the many categories of immune response, the immune system functions as a single entity to defend the host from infections by pathogens.

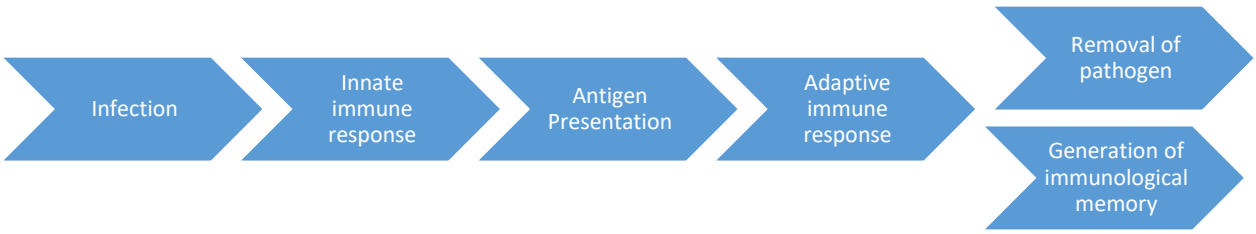


Fig. 2.2: Flow of events involving the immune system

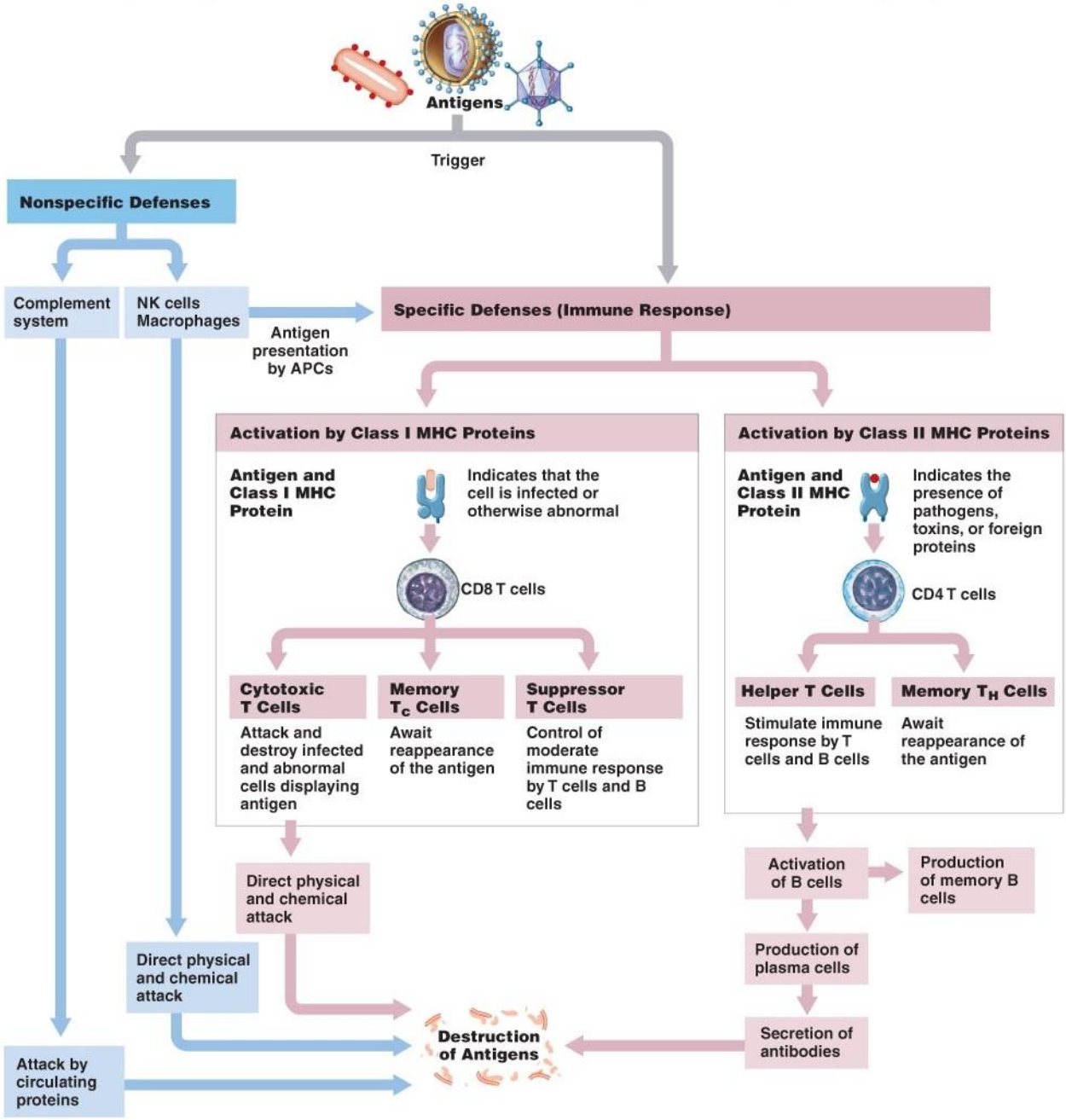


Fig. 2.2: Relationship between innate and adaptive immunity

2.1 INNATE IMMUNITY (NON-SPECIFIC)

- Innate immunity is **genetically determined** and provides broad defences against infection. It is the **first line of defence** against infection and responds the same way for every antigen encounter.

- The innate immune system recognises structures that are shared by various classes of pathogens and are **not present on normal host cells**. Innate immunity functions to:
  (i) kill invading pathogens
  (ii) activate the adaptive immune response

- Innate immune system consists of
  (i) **external** defence
  (ii) **internal** defence

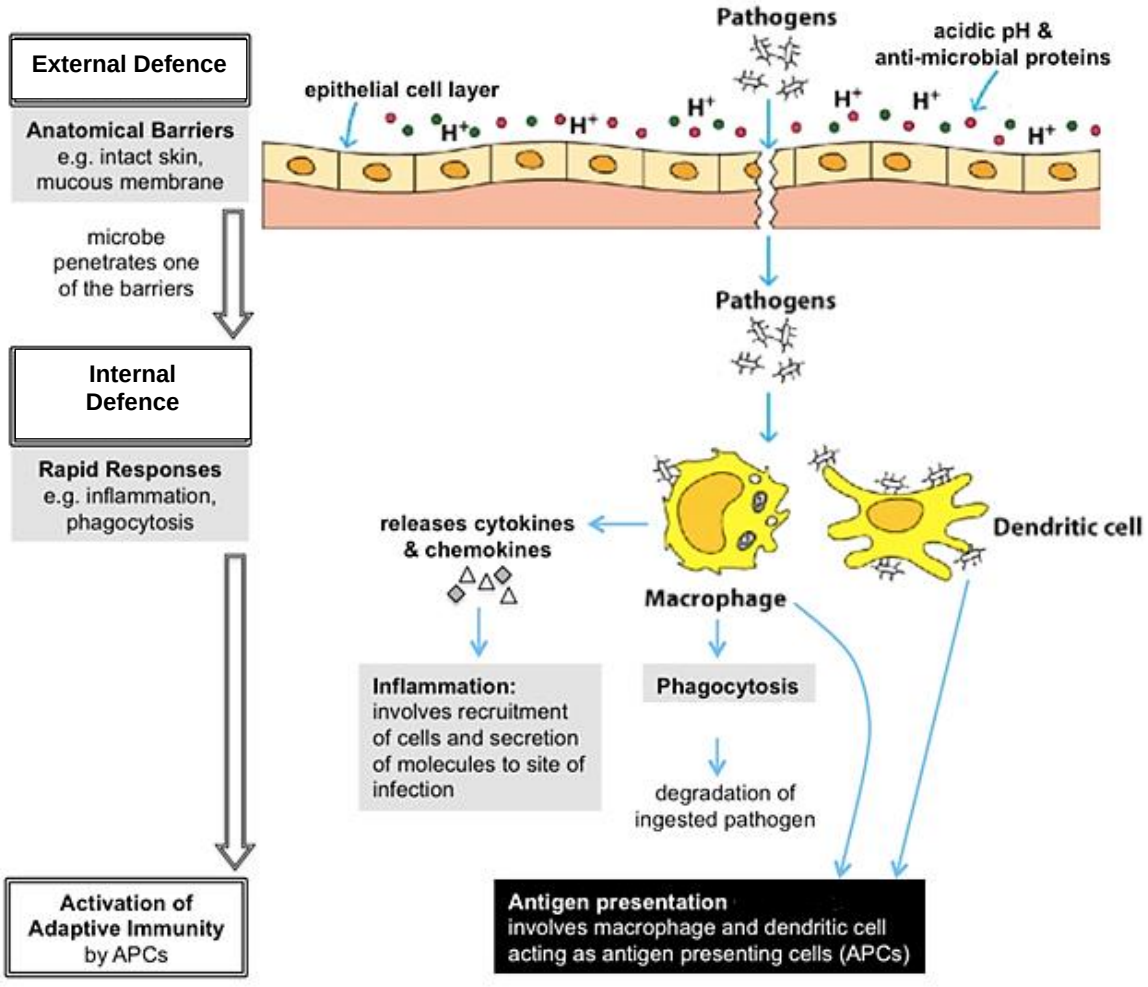


Fig. 2.1.1: Overview of innate immunity

2.1.1 External Defence: Barriers to Infection

- The external defence of the innate immune system begins with the physical, chemical and biochemical barriers below:

Barriers to Infection	Examples	Descriptors
Physical	Skin and mucosa	Intact structures provide a physical barrier to prevent entry of organisms e.g. microorganisms
	Cilia	Hair-like structures found in respiratory tract to propel microorganisms towards the throat where they can be expelled by coughing / swallowing / excretion.
	Mucus	It is sticky and slimy to entrap microorganisms for expulsion of particles on the gut, respiratory tract and genito-urinary tract.
	Coughing and sneezing	Expulsion of air to remove microorganisms.
Chemical & biochemical	Acids	Hydrochloric acid secreted by the stomach is lethal to many bacteria. Lactic and proprionic acid secreted by the commensal bacteria in the vagina resulted in low pH, inhibiting division of many bacteria.
	Fatty acids	Produced by sebaceous glands in the skin, they have antimicrobial properties.
	Lysozyme	Breaks down peptidoglycan in bacterial cell walls, thus damaging and killing the bacteria.
	Anti-microbial peptides	Antimicrobial and antibacterial proteins

2.1.2 Internal Defence: Cellular Defence

- Some pathogens may be able to penetrate the epithelial cell barrier. When this occurs, the **inflammatory response** is activated to recruit cells and other factors from the bloodstream to remove the pathogen.

- The inflammatory response involves the following:

  – **Vasodilation**, which causes increased blood flow to the affected tissue site, hence increasing the supply of cells and proteins

  – **Activation of endothelial cells** lining the blood vessels, which promotes cellular migration into the affected tissue site

  – Increased **vascular permeability**, allowing cells and proteins to enter the affected site

  – Production of **chemotactic factors** to attract cells from the blood into the affected site

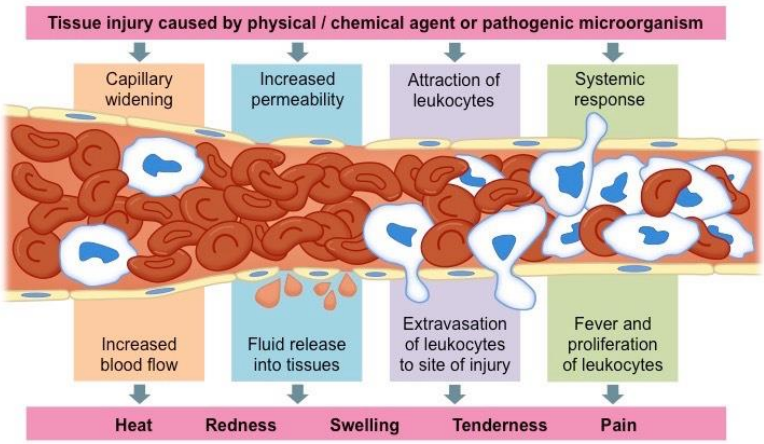


Fig. 2.1.2: Inflammatory response (1)

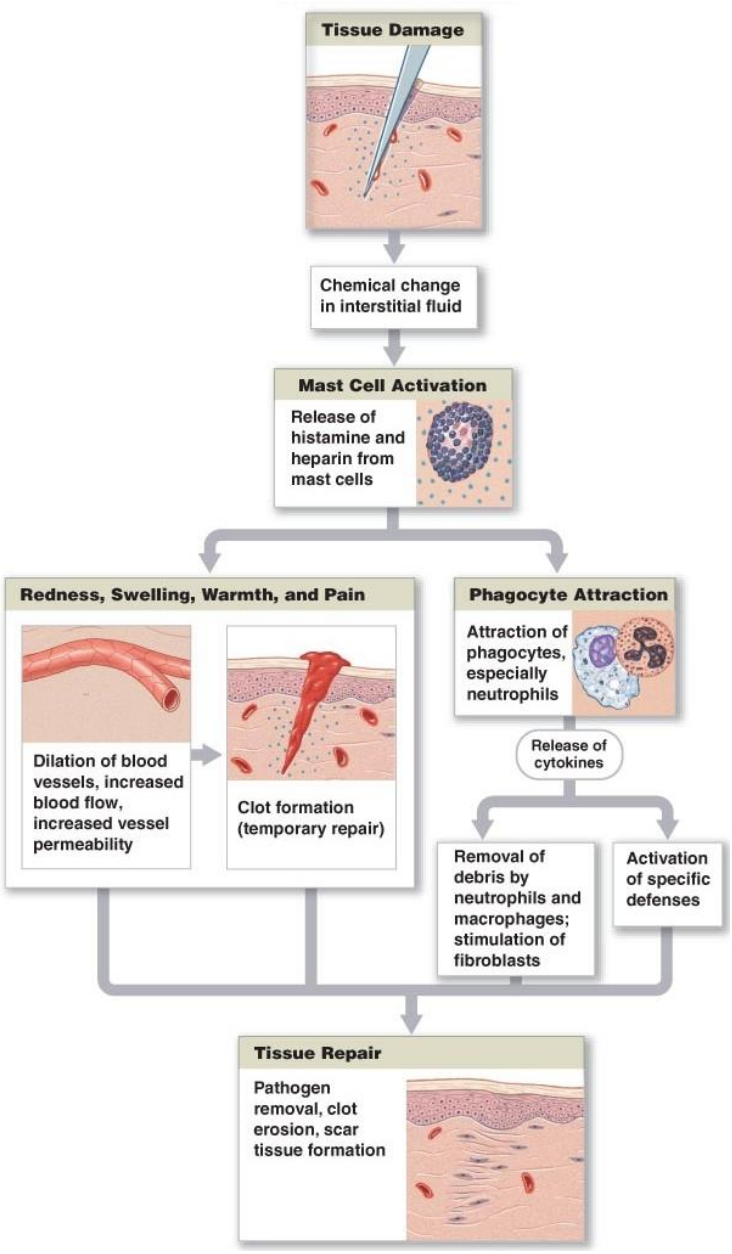
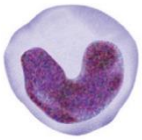
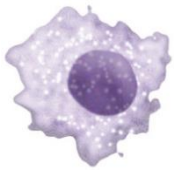
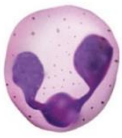
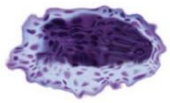
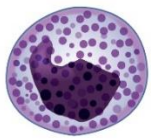
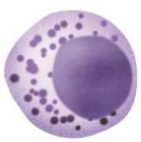


Fig. 2.1.3: Inflammatory response (2)

- The cells below are components of the innate immune system that participates in the inflammatory response.

Cell Type	Location	Function
Monocyte 	Blood	Circulates in the blood stream until it receives specific chemical signals to leave the blood stream to infected tissues and differentiate into macrophages.
Macrophage 	Tissues throughout the whole body	First cells to encounter pathogens, ingest the pathogen via phagocytosis and destructing the pathogen.
Dendritic Cell	Tissues throughout the whole body	
Neutrophil 	Bone marrow, Blood	First cells to be recruited to affected tissue site to participate in the ingestion of the pathogen via phagocytosis and destructing the pathogen.
Eosinophil 	Blood	Phagocytize pathogens coated with antibodies and are also involved in direct killing of pathogens through releasing lytic factors
Mast Cell  **Basophil** 	Tissues throughout the whole body	Release factors e.g. histamine, heparin and proteolytic enzymes and synthesize proteins to cause vasodilation and attract neutrophils to affected site to amplify the inflammatory response
Natural Killer (NK) Cell 	Blood	Recognize and kill infected cells

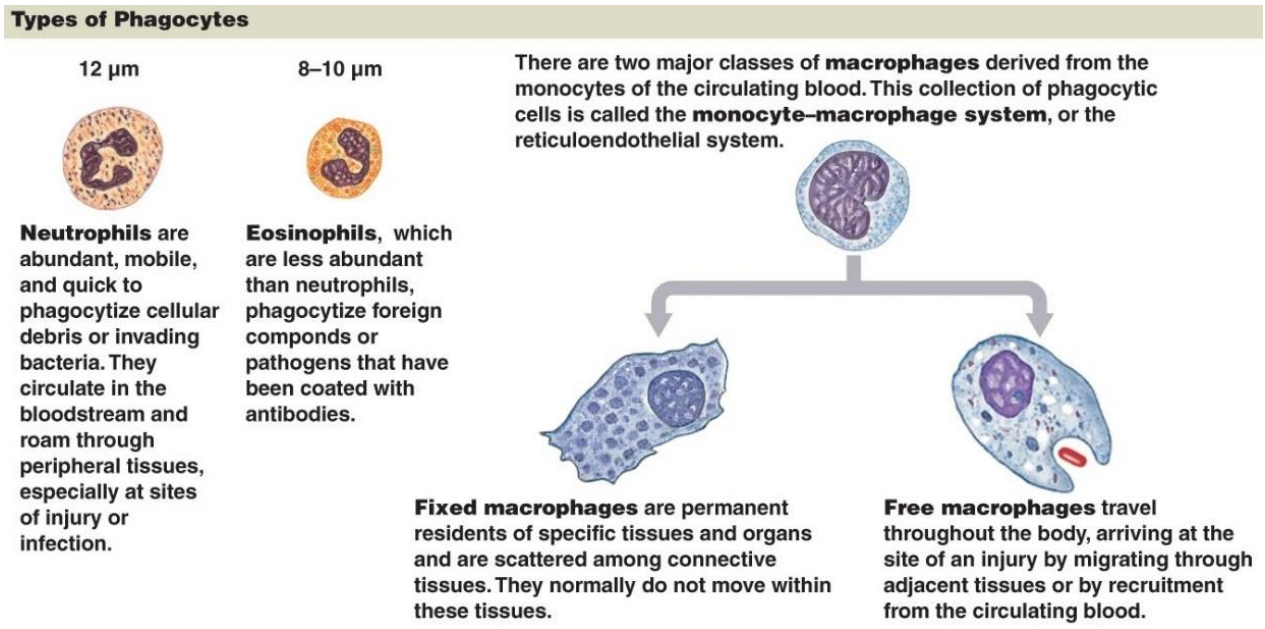
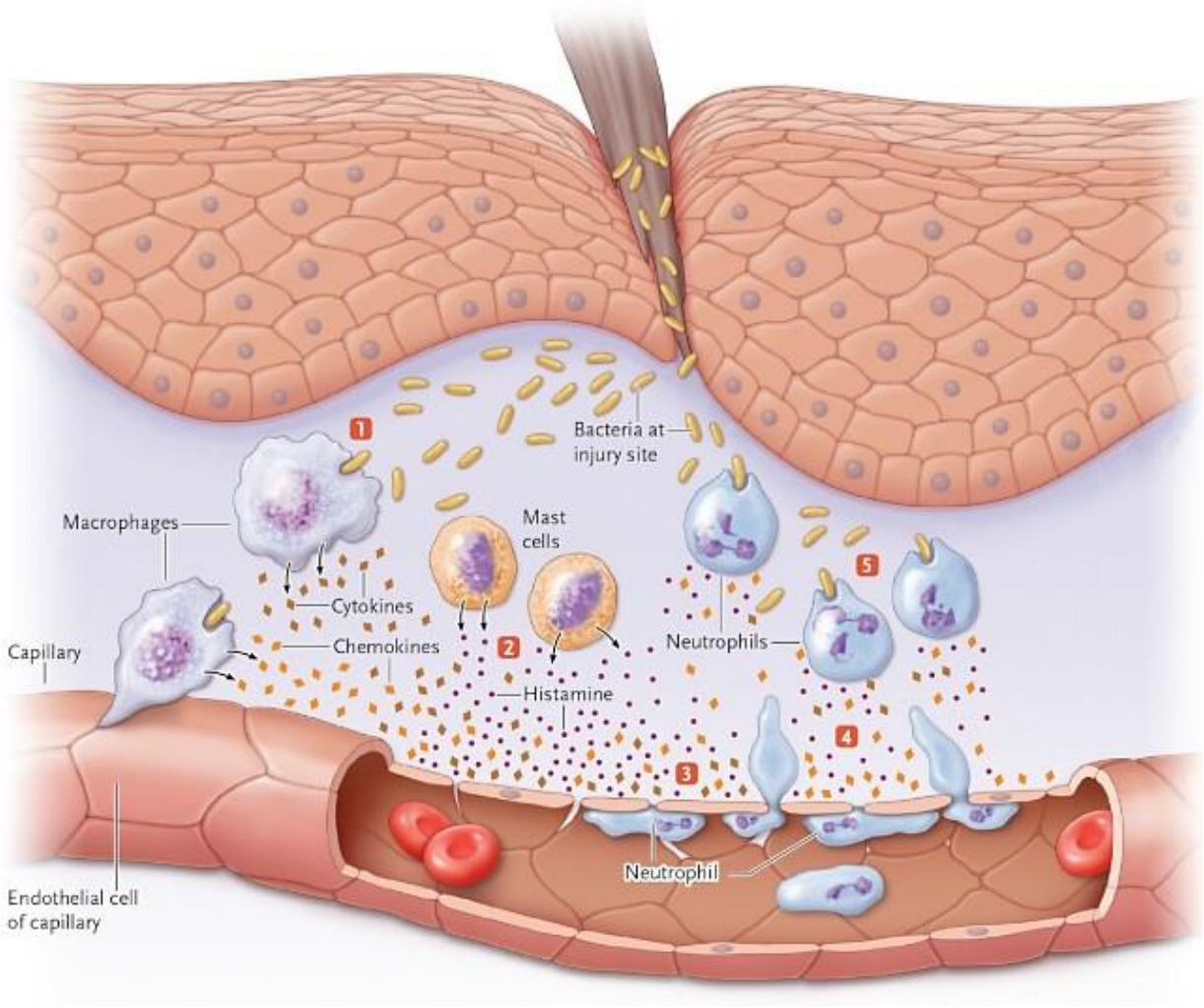


Fig. 2.1.4: Types of phagocytes

CHECKPOINT 1

Match the statements below with the steps in the inflammatory response.



Statement	Step
Chemokines attract neutrophils, which pass between cells of the blood vessel wall and migrate to the infection site	
A break in the skin introduces bacteria, which reproduce at the wound site. Activated macrophages engulf the pathogens and secrete cytokines and chemokines.	
Neutrophils engulf the pathogens and destroy them.	
Histamine and cytokines dilate local blood vessels and increase their permeability. The cytokines also make the blood vessel wall sticky, causing neutrophils to attach.	
Activated mast cells release histamine.	

2.2 ANTIGEN PRESENTATION

Learning Outcome (b)
Outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses

- Antigen presentation serves as the link between the innate and adaptive immune systems.

- **Antigen presentation** is a display of **antigenic peptides** bound to membrane proteins called MHC (major histocompatibility complex) proteins on the **surface of an antigen-presenting cell**. The antigen-presenting cell will then present the antigenic peptides to cells of the adaptive immune system, activating them.

- **Antigen-presenting cells (APCs)** are cells that take up antigens and process them into short peptides, before presenting the peptides on MHC to lymphocytes for recognition. Examples of APCs include **dendritic cells**, **macrophages** and **B cells**.

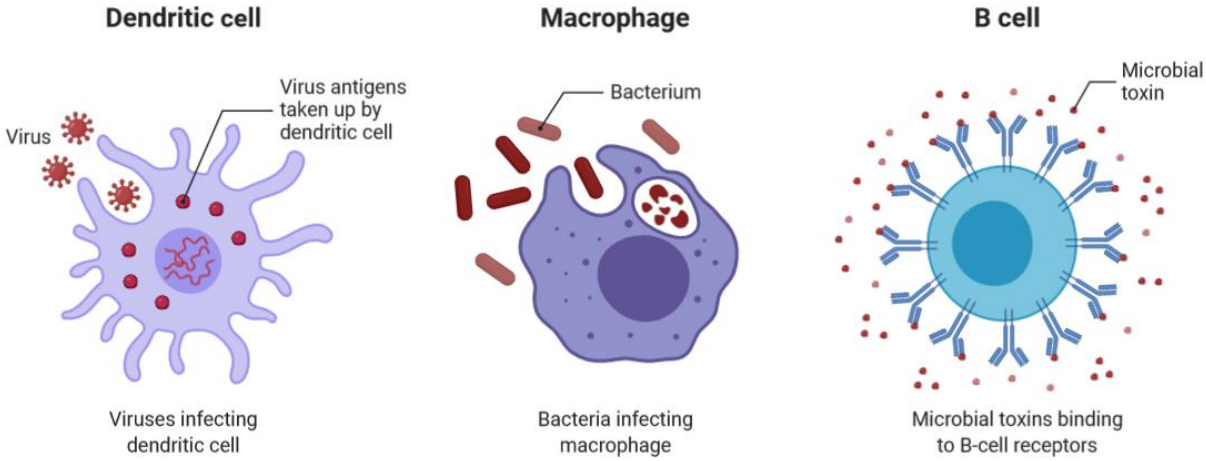


Fig. 2.2.1: Antigen-presenting cells

Steps involved in Antigen Processing

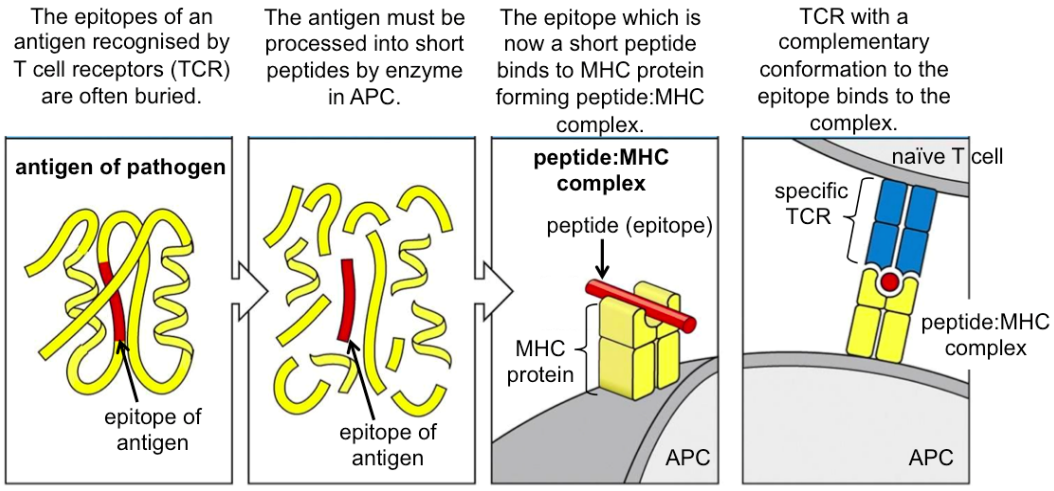


Fig. 2.2.2: Antigen processing (1)

1) The APC engulfs the pathogen via **phagocytosis**, enclosing the pathogen in a phagosome. The phagosome **fuses with the lysosome**, forming a phagolysosome.

2) The antigen is processed by **digesting the antigen into smaller antigenic peptides**, by hydrolytic enzymes in the lysosome.

3) **MHC receptors** are synthesised by ribosomes on the rough endoplasmic reticulum, and modified in the Golgi apparatus.

4) A transport vesicle from the Golgi apparatus containing the MHC receptor fuses with the phagolysosome. The **antigenic peptides** will then bind to the **MHC receptor**, forming a **antigenic peptide:MHC complex**.

5) The vesicle containing the antigenic peptide:MHC complex is transported to the cell surface membrane. It then fuses with the cell surface membrane, embedding the **antigenic peptide:MHC complex in the cell surface membrane**. The antigenic peptide can now be presented to **naïve T cells** to activate these cells.

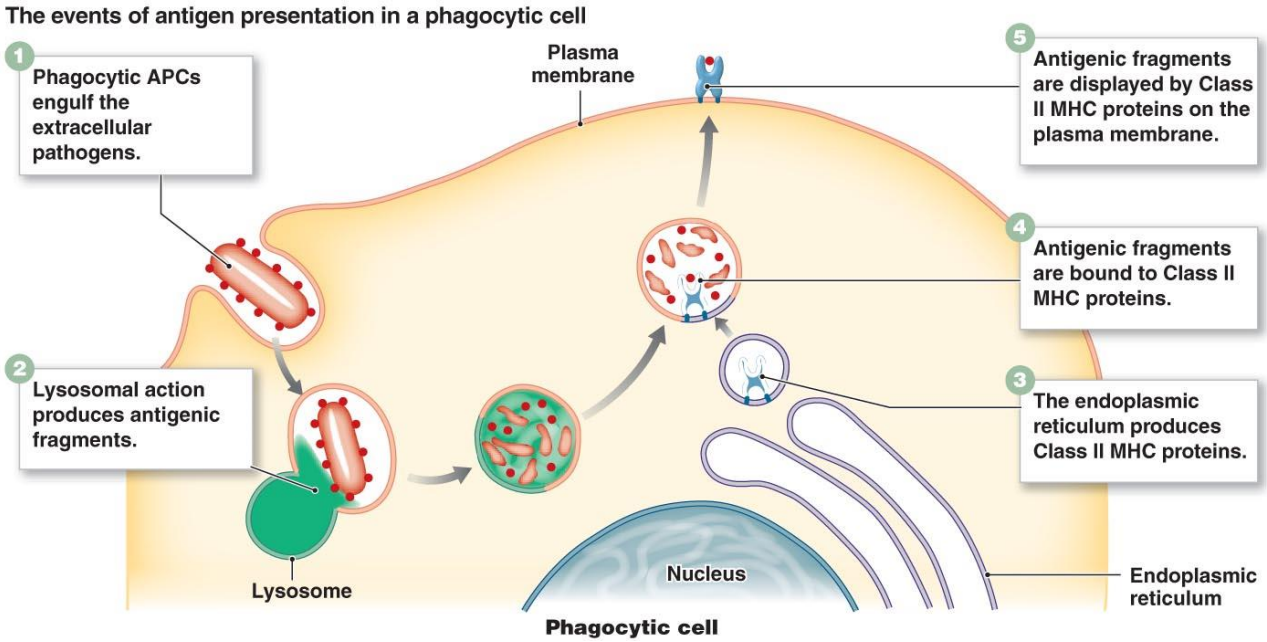


Fig. 2.2.3: Antigen processing (2)

2.3 ADAPTIVE IMMUNITY (SPECIFIC)

Learning Outcome (a)
Describe the specific (adaptive) and non-specific (innate) immune systems including active and passive, natural and acquired immunity.

- When the pathogen non-specific (innate) immune response fails to eliminate the pathogen, the specific (adaptive) immune response occurs.

- Specific adaptive immunity involves the **cell-mediated** and **humoral response**.

  – **Cell-mediated immune response** is mediated by **T cells**. This protects against **intracellular pathogens** by killing cells infected by these pathogens.

  – **Humoral immune response** is mediated by **antibodies** that are secreted by **plasma cells** (activated **B cells**). This protects against **extracellular pathogens** and toxins secreted by pathogens into the extracellular space.

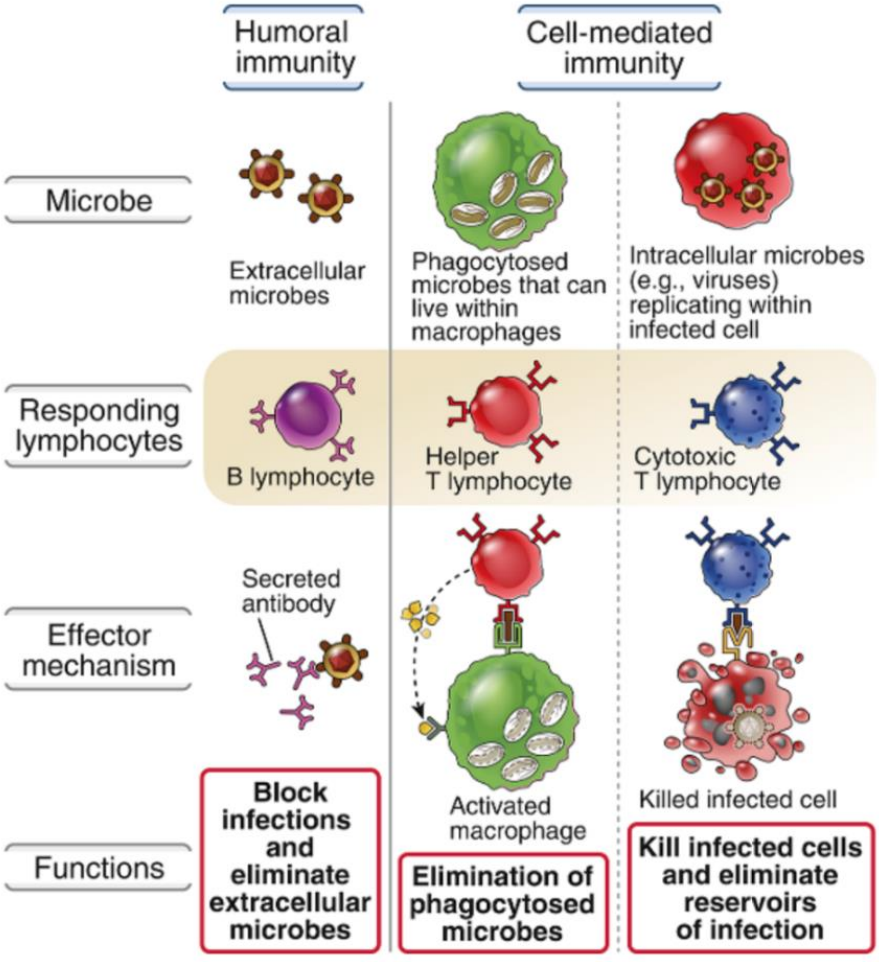


Fig. 2.3.1: Humoral and cell-mediated immunity

- The adaptive immune response involves B and T cells.

Development and Maturation of B and T cells

- B and T cells originate from a **common lymphoid progenitor cell** in the **bone marrow**.
  – Immature B cells will continue to mature in the **bone marrow**, after which they migrate to the **spleen** to complete its maturation, forming mature **naïve B cells**.
  – Immature T cells will leave the bone marrow and migrate to the **thymus** to complete the maturation process, forming mature **naïve T cells**.

- During the maturation of B and T cells in the bone marrow and thymus respectively, the following processes occur:
  (i) **Gene rearrangement** to produce **a wide diversity of B cell and T cell receptors** (immunoglobulins / antibodies) on B and T cells, which allows these lymphocytes to recognise and bind to specific antigenic epitopes.
  (Refer to Section 3.1: Generation of Antibody Diversity)
  (ii) Immature lymphocytes are tested for **self-reactivity**, where lymphocytes with receptors complementary to antigenic epitopes of the body's own molecules are removed by apoptosis, while lymphocytes that survive undergo maturation.

- After the production of mature naïve B cells and T cells, these lymphocytes remain inactive and are **circulated in the blood and lymph**, or reside in secondary lymphoid organs. When a specific antigen binds to the receptors of the lymphocytes, the lymphocytes are **activated** to **divide and differentiate to form effector B and T cells.**

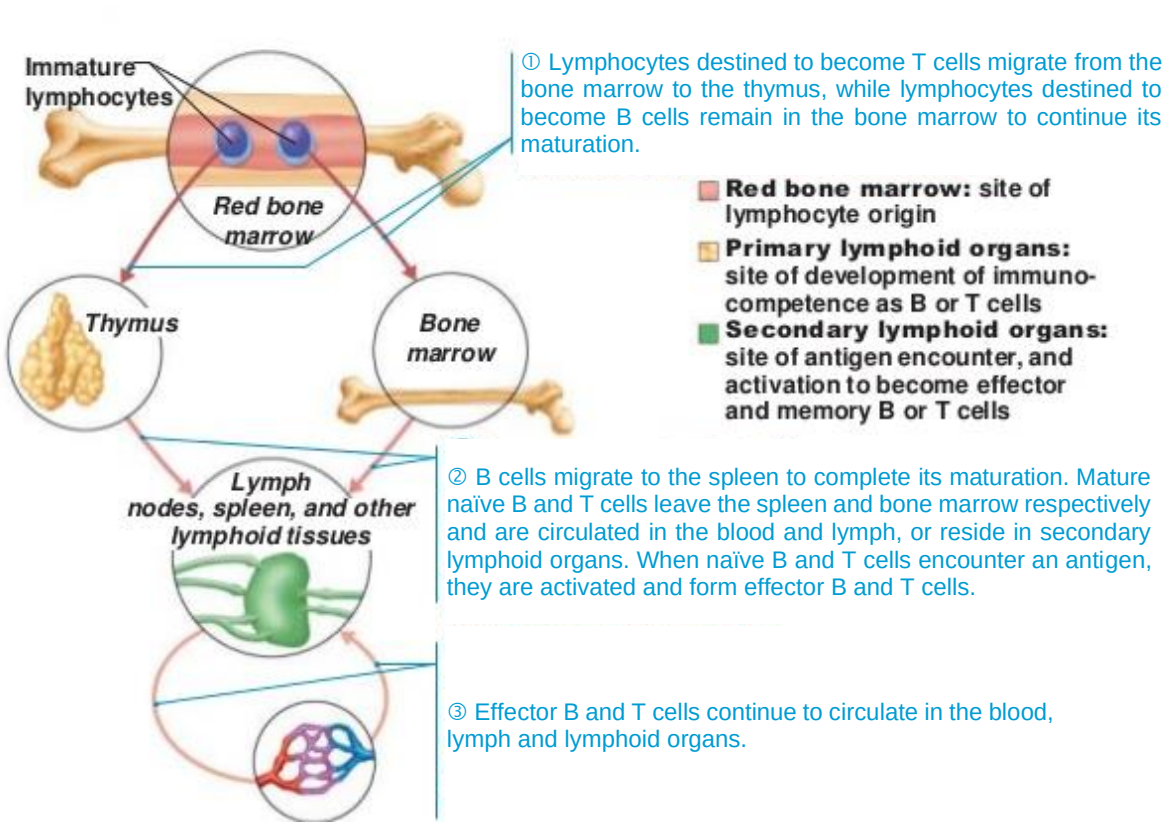


Fig. 2.3.2: Maturation and activation of B and T cells

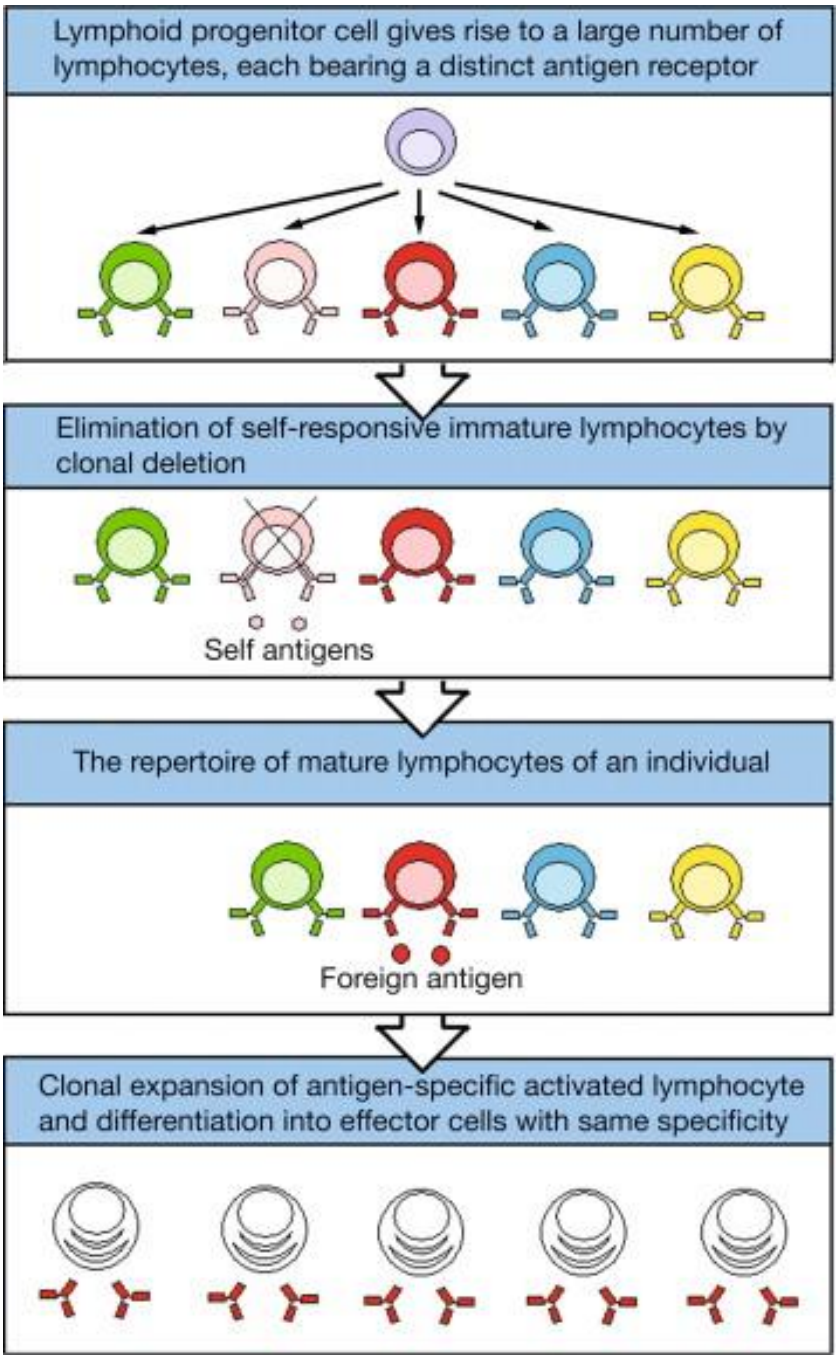


Fig. 2.3.3: Development and maturation of lymphocytes with a wide variety of receptors, followed by activation of specific lymphocyte with receptor specific for antigen

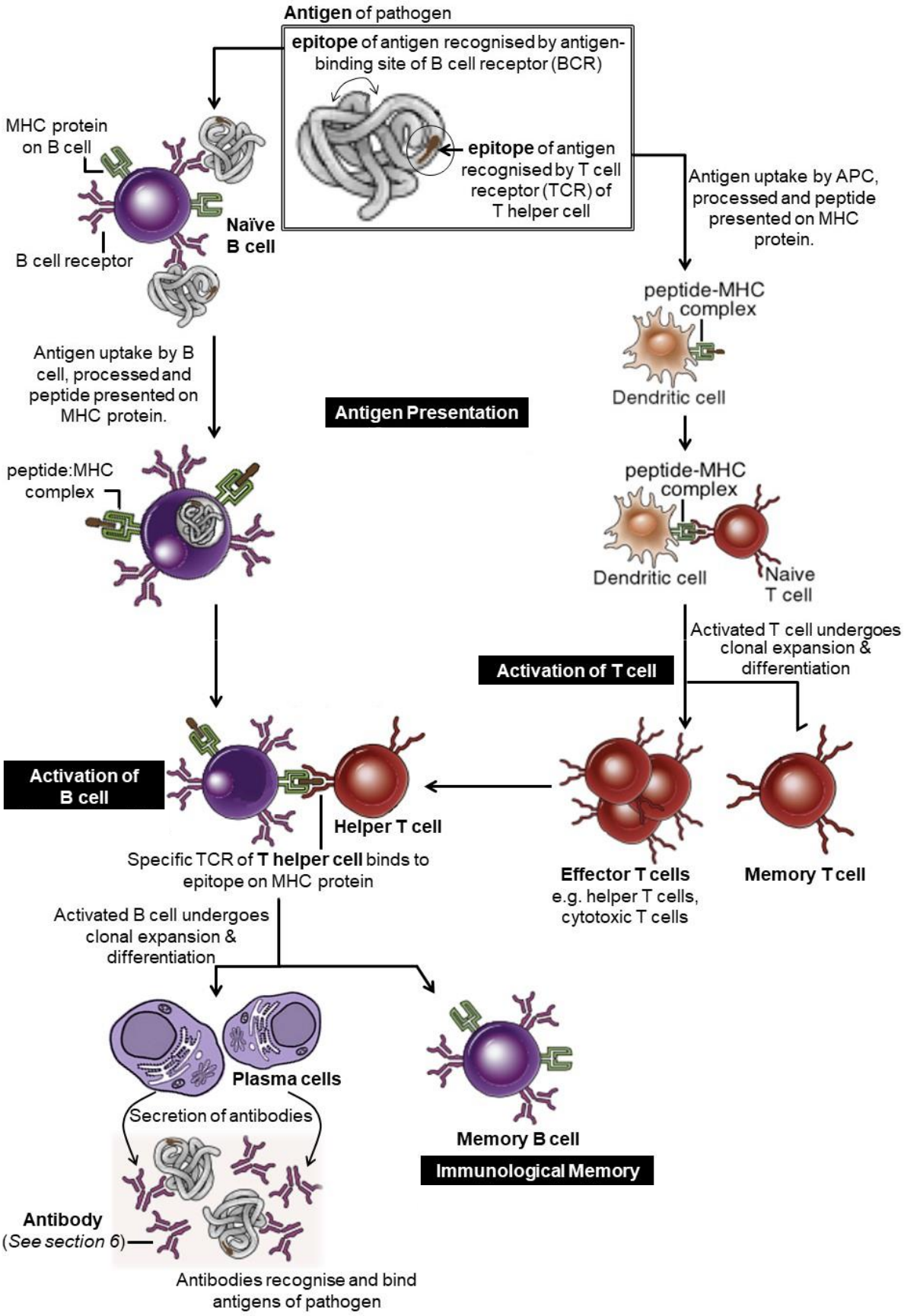


Fig. 2.3.4: Overview of activation of naïve B and T cells

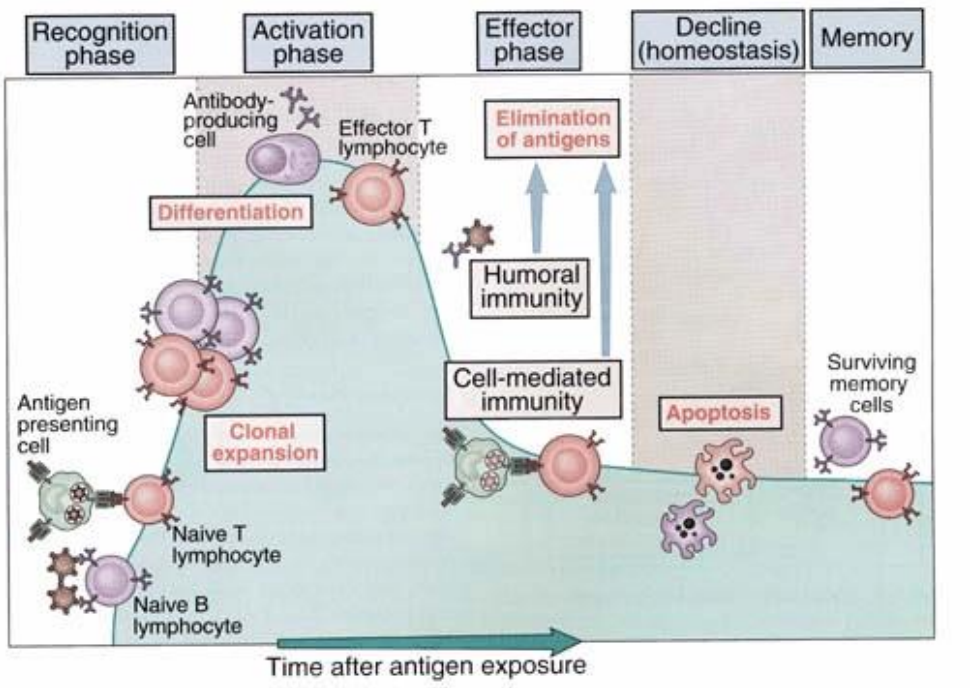


Fig. 2.3.5: Stages of adaptive immune response

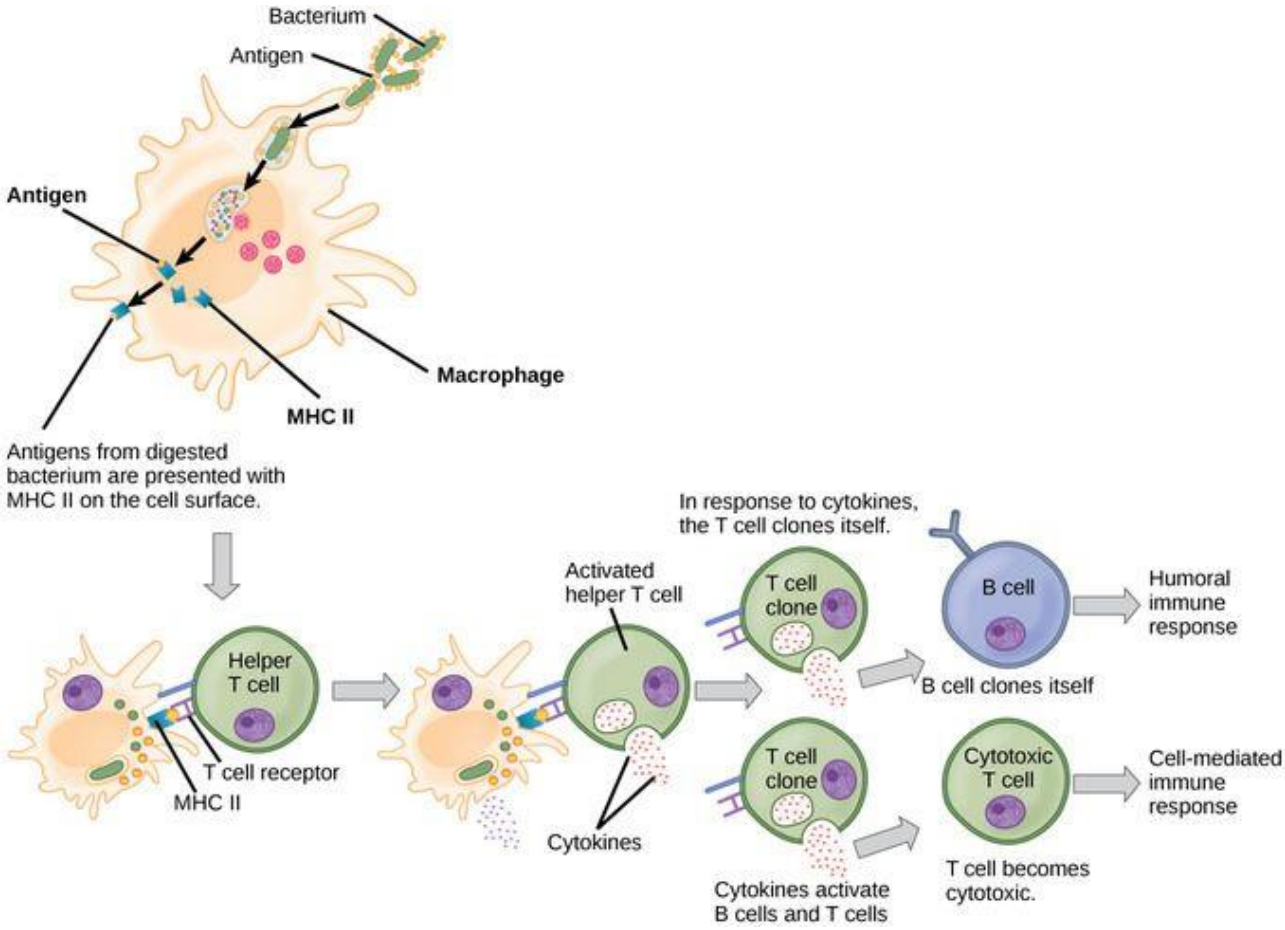


Fig. 2.3.6: Antigen processing and presentation to activate naïve B and T cells in adaptive immune response

2.3.1 Cell-mediated Immunity

Learning Outcome (b)
Outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses

Activation of T Cells

- During antigen presentation, antigen-presenting cells (APCs) present the antigenic peptide to mature naïve T cells. Naïve T cells with **specific T cell receptors (TCRs)** will recognise and bind to the antigenic peptide:MHC complex on APCs.

- Activation of naïve T cells require the following signals:

  – Signal 1: Binding of antigenic peptide:MHC complex with TCR
  – Signal 2: Binding of co-stimulatory molecules
  – Signal 3: Secretion of cytokines by APC

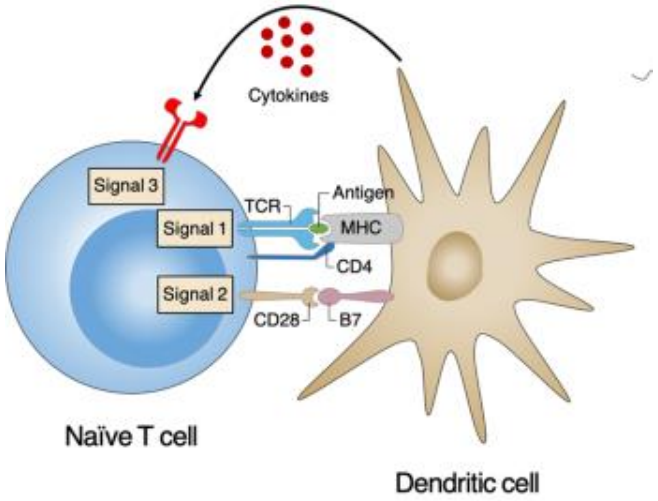


Fig. 2.3.1.1: Activation of naïve T cells requires three signals

- The naïve T cell will be activated to proliferate, producing large numbers of genetically identical daughter cells, via **clonal selection** and **expansion**. These cells then undergo **differentiation** to form **effector T cells**:

  – Helper T cells (T_H), which contain CD4 co-receptors
  – Cytotoxic T cells (T_C), which contain CD8 co-receptors
  – Memory T cells (either T_H or T_C cells)

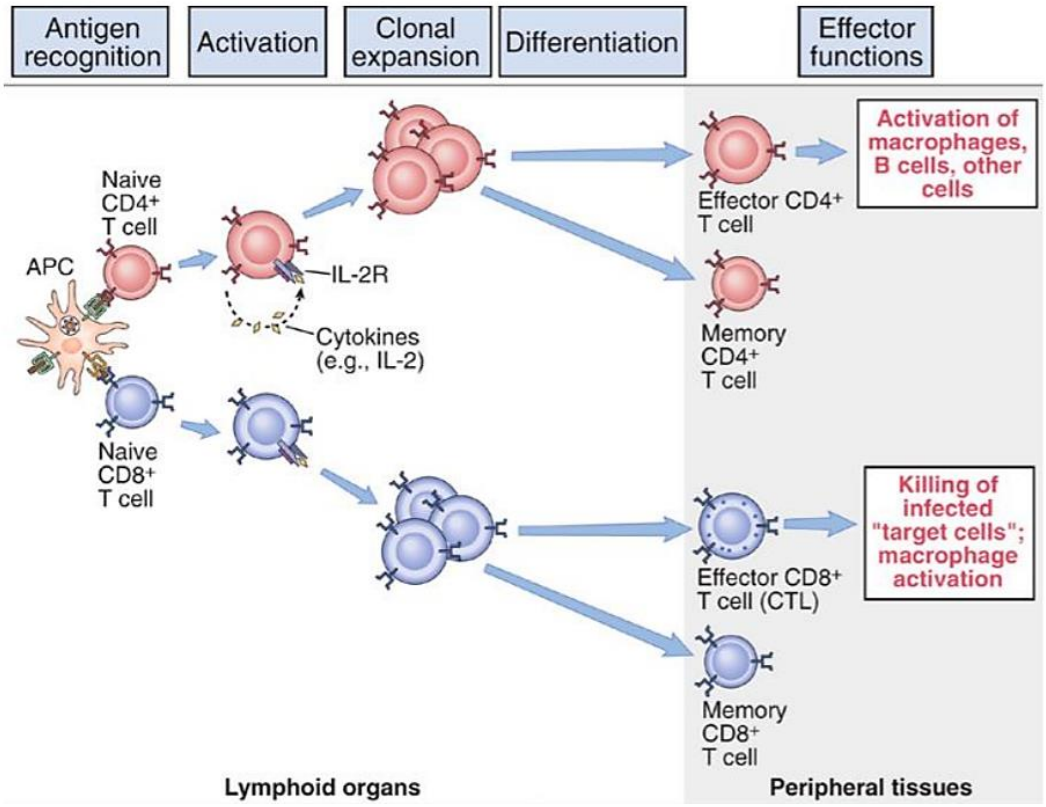


Fig. 2.3.1.2: Activation of naïve T cells to form effector cells

Role of CD8+ Cytotoxic T Cells

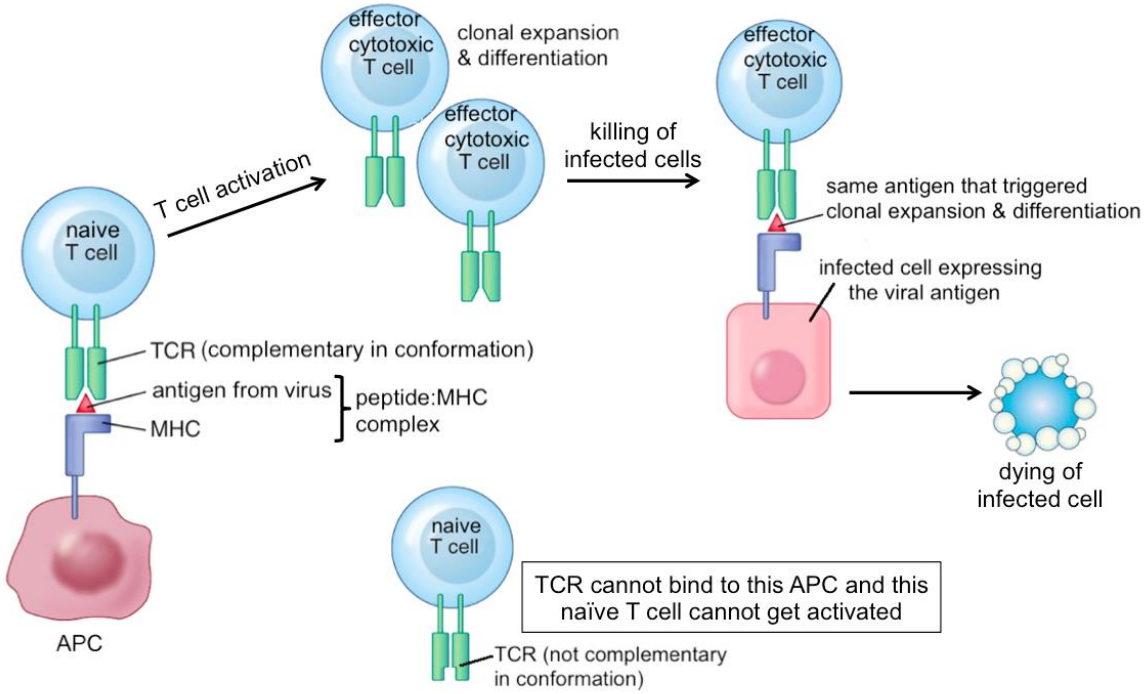


Fig. 2.3.1.3: Activation of naïve T cells, which divide and differentiate to form effector cytotoxic T cells that kill infected cells

- Cytotoxic T cells kill **cells infected with viruses or other intracellular pathogens**. These infected cells display an **antigenic peptide:MHC complex**, allowing the **specific T cell receptor** of the cytotoxic T cell to recognise and bind. Cytotoxic T cells then kill infected cells by releasing:

  – **perforins** – molecules which make pores in the cell membrane of the infected cell
  – **granzymes** – proteases which activate apoptosis in the target cell

- Cytotoxic T cells can also kill tumour cells.

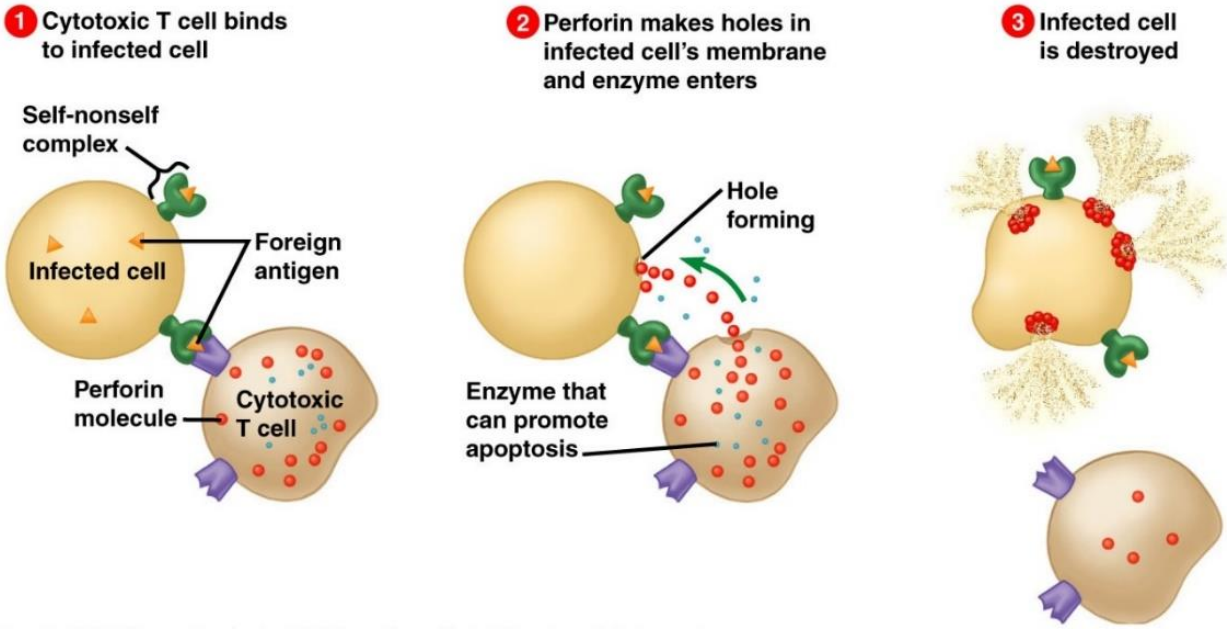


Fig. 2.3.1.4: Action of cytotoxic T cells on infected cells

Role of $CD4^+$ Helper T Cells

- Helper T cells produce **cytokines** (e.g. interleukins, IL), which are small proteins that enable communication between cells of the immune system. Secretion of cytokines result in:

  – **Activation of B cells** to secrete antibodies *(Refer to Section 2.3.2: Humoral Immunity)*

  – **Activation of cytotoxic T cells**, allowing the cells to proliferate and differentiate to form effector cytotoxic T cells

  – **Activation of macrophages**, stimulating phagocytic activity and effectiveness at presenting antigens to T cells

  – **Recruitment of neutrophils** to sites of infection and enhancing their responses against pathogens

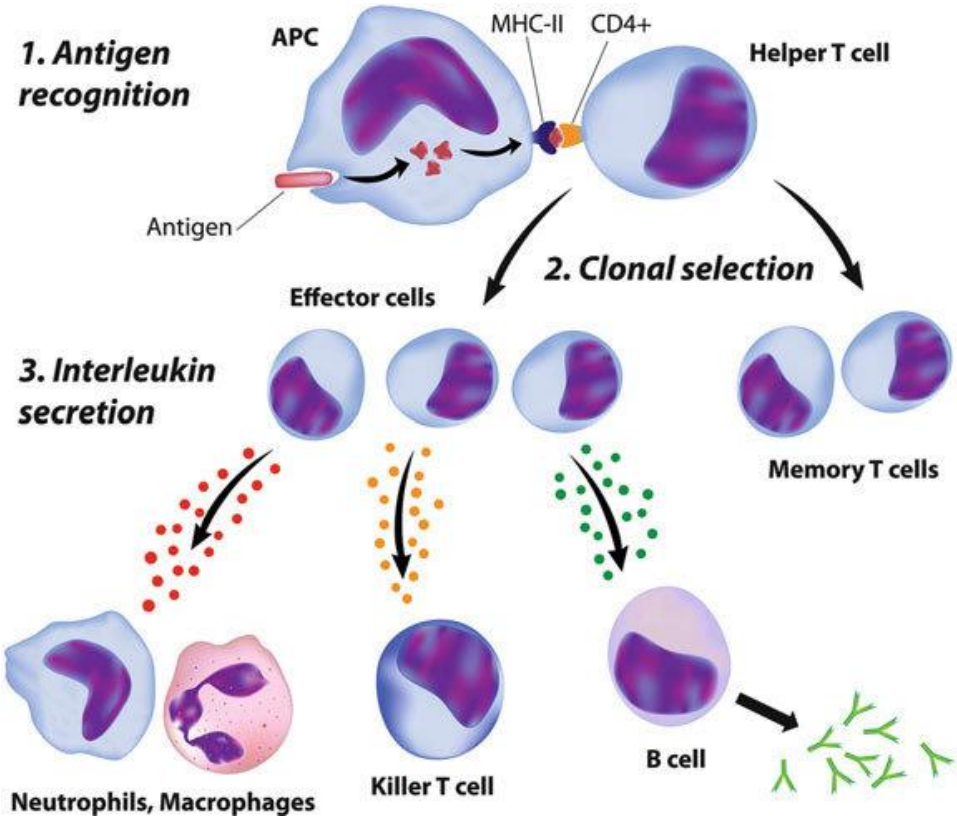


Fig. 2.3.1.5: Roles of helper T cells

2.3.2 Humoral Immunity

Learning Outcome (b)
Outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses

Activation of B cells

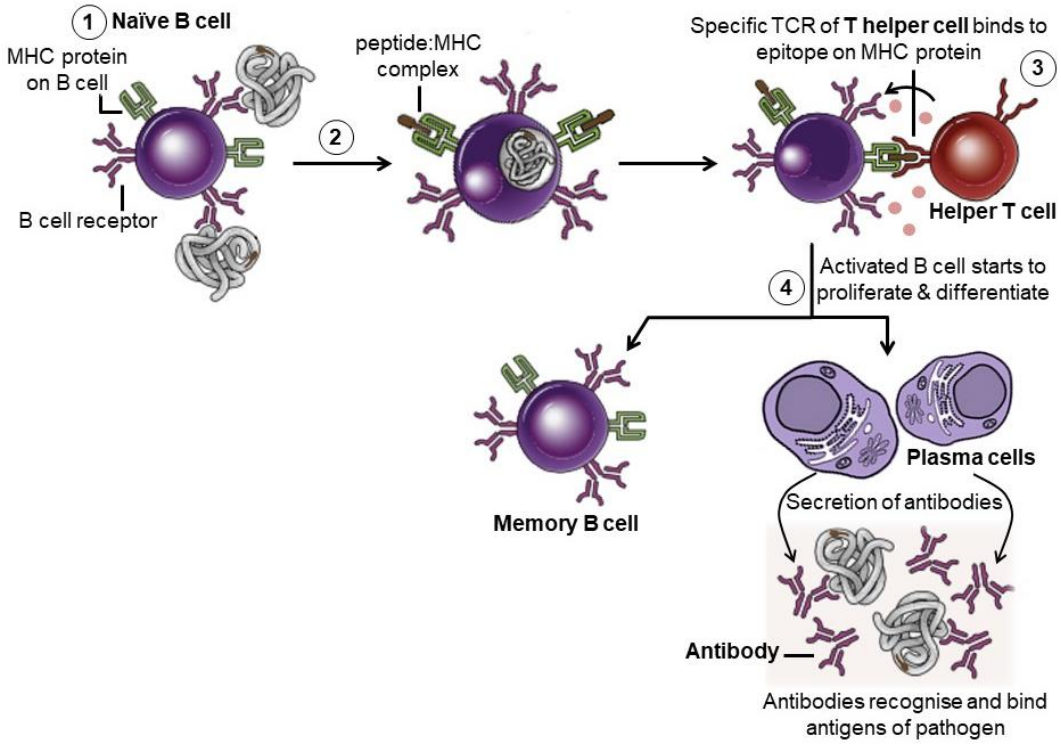


Fig. 2.3.2.1: Activation of naïve B cells to form effector cells

(1) Each naïve B cell has a specific **B cell receptor (BCR)** on its cell surface that can recognise and bind a specific antigenic epitope.

(2) The antigen is taken up via endocytosis, **processed** and **presented** via **antigenic peptide:MHC complex**.

(3) The specific **T cell receptor** of the helper T cell is able to recognise and bind to the antigenic peptide presented.

(4) Interaction between the helper T cell and B cell stimulates **cytokine secretion** from the helper T cell, activating the B cell to undergo **clonal expansion** and **differentiation** into effector cells. **Affinity maturation** and **class switching** also occurs to produce effector B cells with antibodies with high affinity for the antigen *(Refer to Section 3.2: Generation of Antibody Diversity)*. Effector B cells include:

   (i) **Antibody-secreting plasma cells**, which are short-lived and secrete antibodies for a specific period of time before undergoing apoptosis.

   (ii) **Memory B cells**, which are long-lived and can survive long after an initial infection has been eliminated.

- Antibodies secreted by plasma cells protect the body against extracellular pathogens and toxins secreted by pathogens into extracellular space. *(Refer to Section 3.1: Structure & Function of Antibodies)*

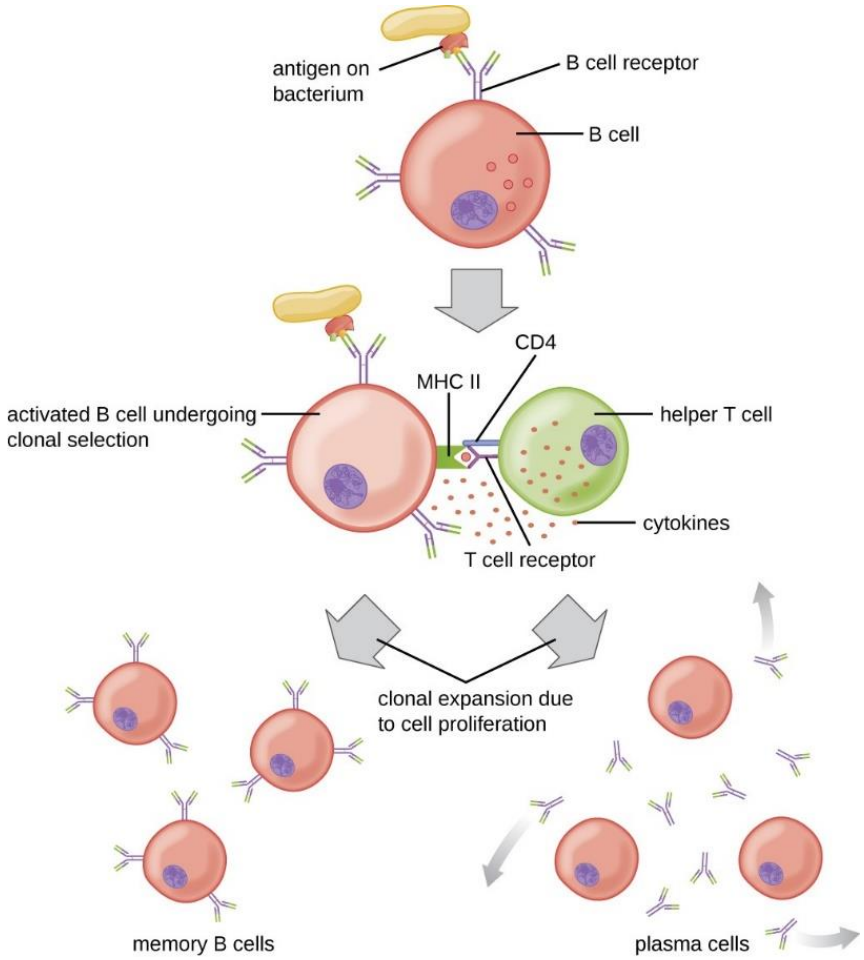


Fig. 2.3.2.2: Activation of naïve B cells to form effector cells (2)

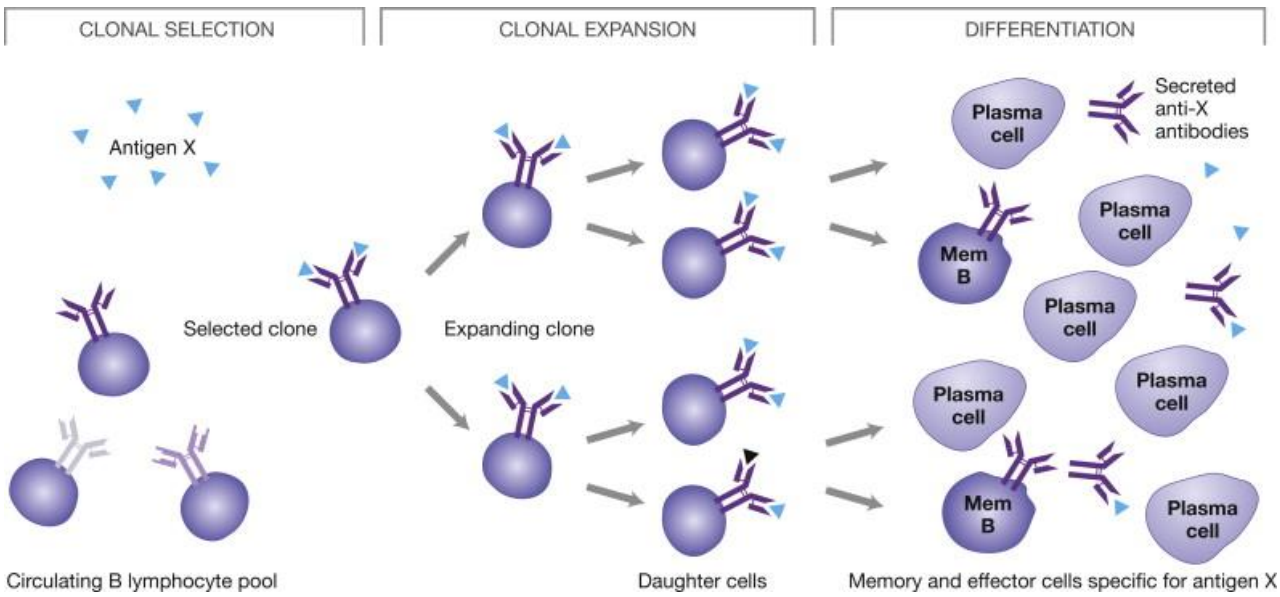


Fig. 2.3.2.3: Clonal selection and expansion of B cells, and differentiation to form effector cells

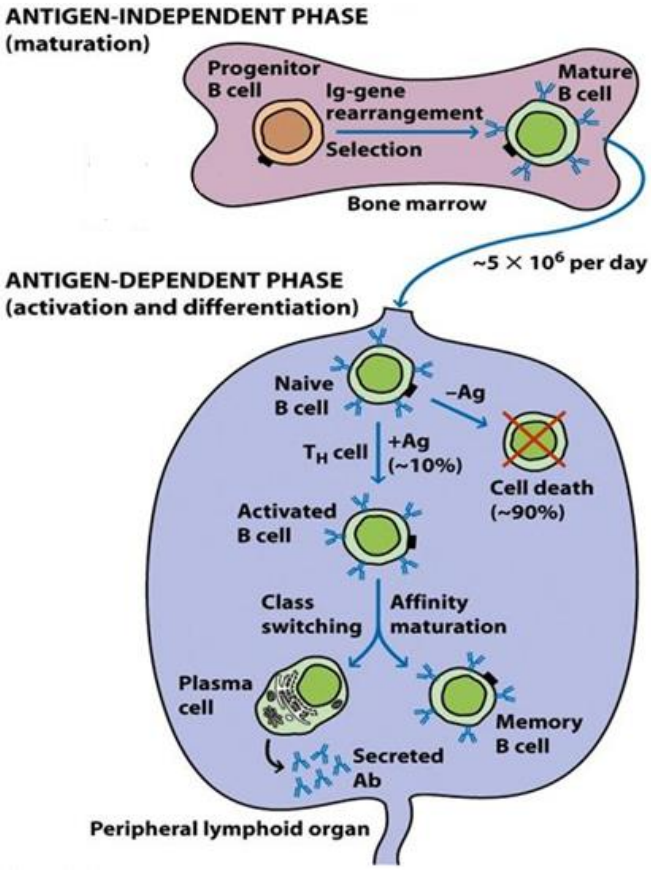


Fig. 2.3.2.4: Maturation, activation and differentiation of B cells

CHECKPOINT 2.1

Fill in the blanks to summarise lymphocyte development.

lymphocytes involved in adaptive immune response

before meeting foreign antigen

_____B cells

_____T cells

encountered specific foreign antigen

_____ B cells

_____T cells

divide and differentiate

_____ B cells

_____ T cells

_____ ***cells***

B cells

T cells (T_H)

T cells

secrete Ig

repeat encounter with same antigen, differentiation occurs

T cells (T_C)

T cells

repeat encounter with same antigen, differentiation occurs

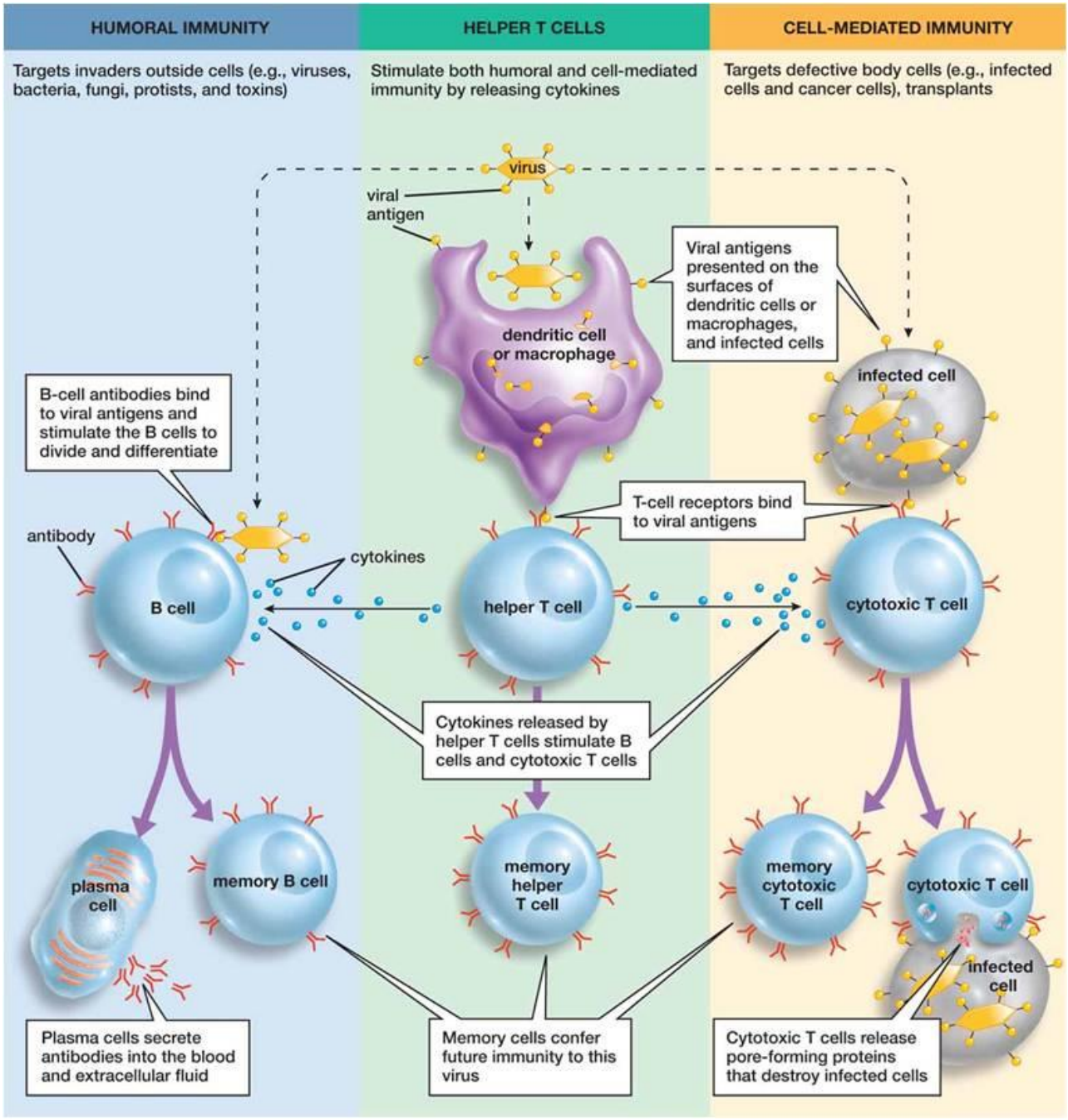


Fig. 2.3.2.5: Overview of cell-mediated and humoral immunity

CHECKPOINT 2.2

Fill in the blanks with the correct word.

(1) Outline the roles of antigen presenting cells

- Intake of _____ into the cell, degrade the antigen into smaller _____ and present the antigenic peptide on _____ to bind to the _____ helper T cell or _____ cytotoxic T cell.
- Express _____ molecules to bind to the T cells.
- Secretes _____ to result in T cell proliferation and differentiation into effector T cells.

(2) Outline the roles of T lymphocytes

- Mature T lymphocytes can be activated by an _____ cell or an effector CD4 helper T cell to result in its division and differentiation into an effector CD4 helper T cell or CD8 cytotoxic T cells respectively.
- CD4 helper T cells secrete _____ to activate B cells to divide and differentiate into plasma cells and memory B cells, and activate CD8 _____ T cell.
- CD8 cytotoxic T cells participate in direct killing of infected cells by causing cell death through cellular _____ or_____.

(3) Outline the roles of B lymphocytes

- Mature B lymphocytes have surface immunoglobulins capable of binding to antigens and surface receptors e.g. _____ that can bind to helper T cells.
- Can be stimulated by cytokines from effector helper T cells to divide and differentiate into _____cells and _____cells.

3 ANTIBODIES

3.1 STRUCTURE AND FUNCTION OF ANTIBODIES

Learning Outcome (c)
Explain the relationship of the molecular structure of antibodies to their functions, using immunoglobulin G, IgG, as an example.

- Antibodies (also known as immunoglobulins) are globular glycoproteins with the ability to recognize foreign molecules on the surface of pathogens or soluble antigens.
- There are five different classes of immunoglobulins IgM, IgG, IgA, IgE and IgD. IgG is the predominant antibody in blood serum.

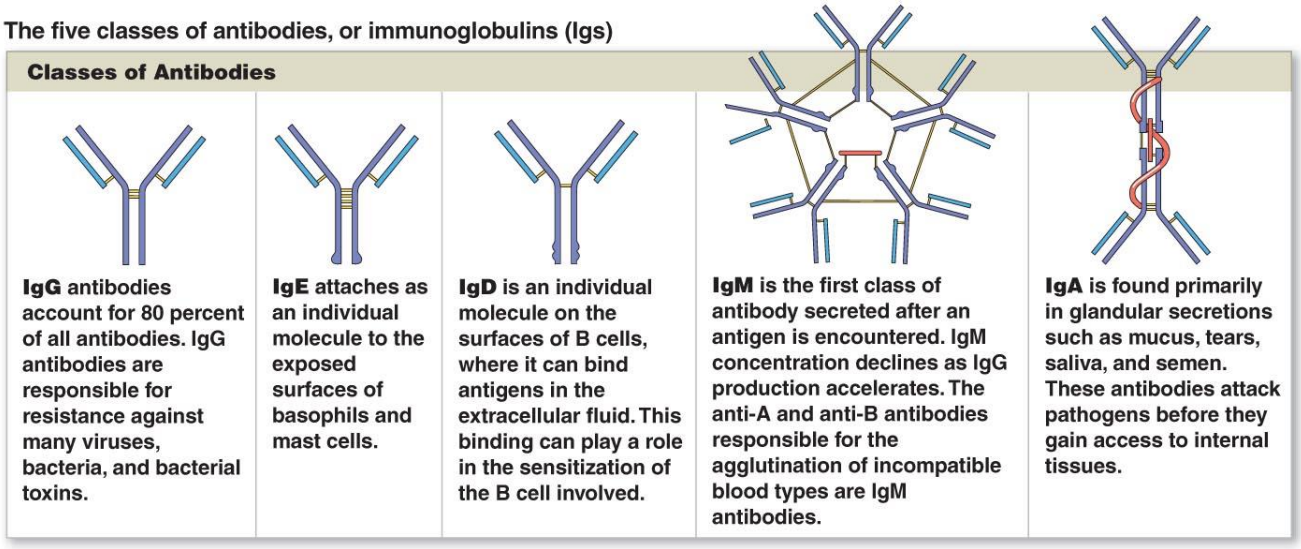


Fig. 3.1.1: Types of immunoglobulins

Structure of Immunoglobulin G (IgG)

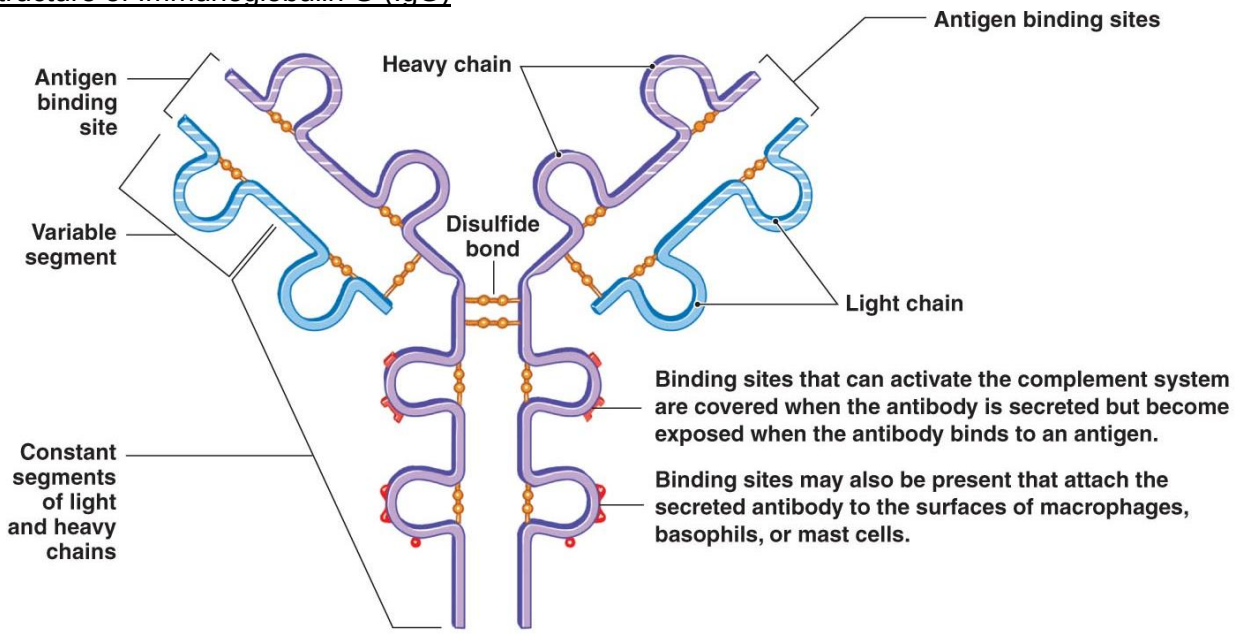


Fig. 3.1.2: Structure of IgG

- IgG is a **globular protein** with a **quarternary structure**.
  - It consists of **four polypeptide chains**, with **two identical heavy (H) chains** and **two identical light (L) chains**. The four chains are held together via inter-chain disulfide bonds and non-covalent interactions.
- Each heavy and light chain consists of the **variable (V) and constant (C) regions**.
- The variable regions of the heavy and light chain (**V_H and V_L** respectively) are brought together to form the **antigen-binding site**, which has a 3D conformation complementary to a specific antigenic epitope. Hence, each antibody has **two identical antigen-binding sites**.

- Each antibody has two **Fab (fragment of antigen binding)** portions and one **Fc (fragment crystallisable)** portion. Fab and Fc portions are joined at the **hinge region**, which is a short sequence of amino acid residues.
  - The **Fab portion** contains the antigen-binding site.
  - The **Fc portion** allows interaction with cell surface receptors (Fc receptors) on other immune cells (e.g. macrophages, natural killer cells).
  - The **hinge region** allows the antibody molecule to be **flexible**. This allows the Fab portion to adopt a wide range of angles, enabling the antibody to bind to antigens spaced at variable distances apart.

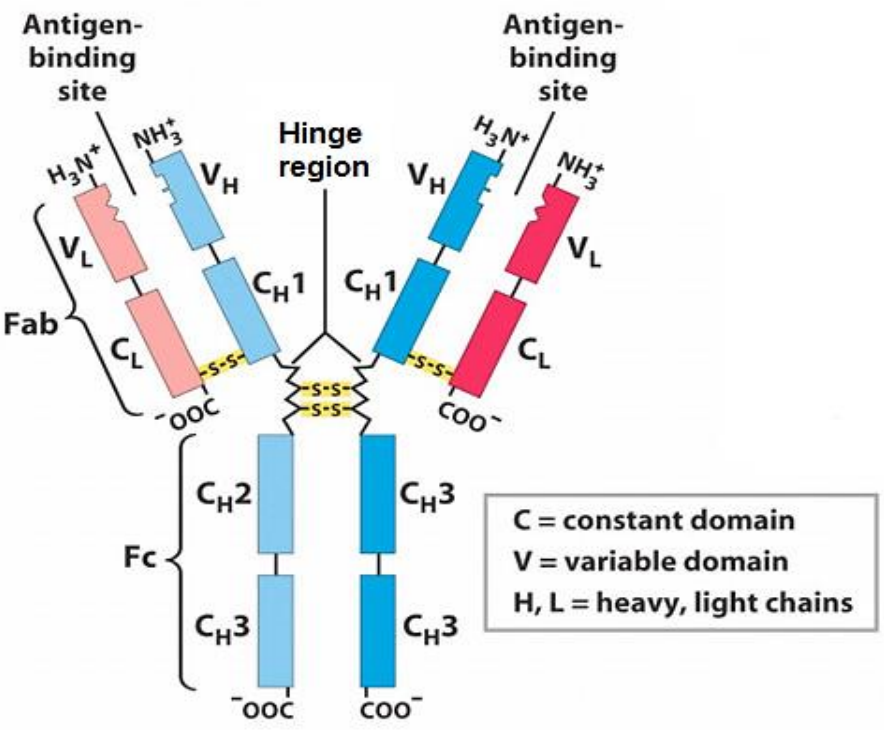
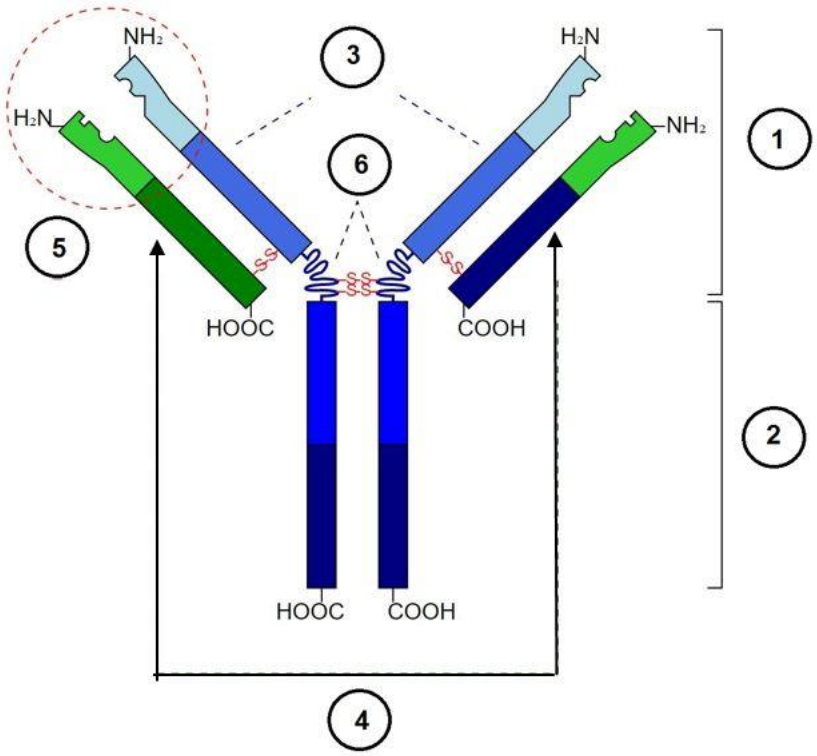


Fig. 3.1.3: Structure of IgG (2)

CHECKPOINT 3

(1) Identify the regions labelled 1 – 6.



(2) Draw the structure of an antigen that can possibly be bound to this immunoglobulin.

Functions of Immunoglobulin G (IgG)

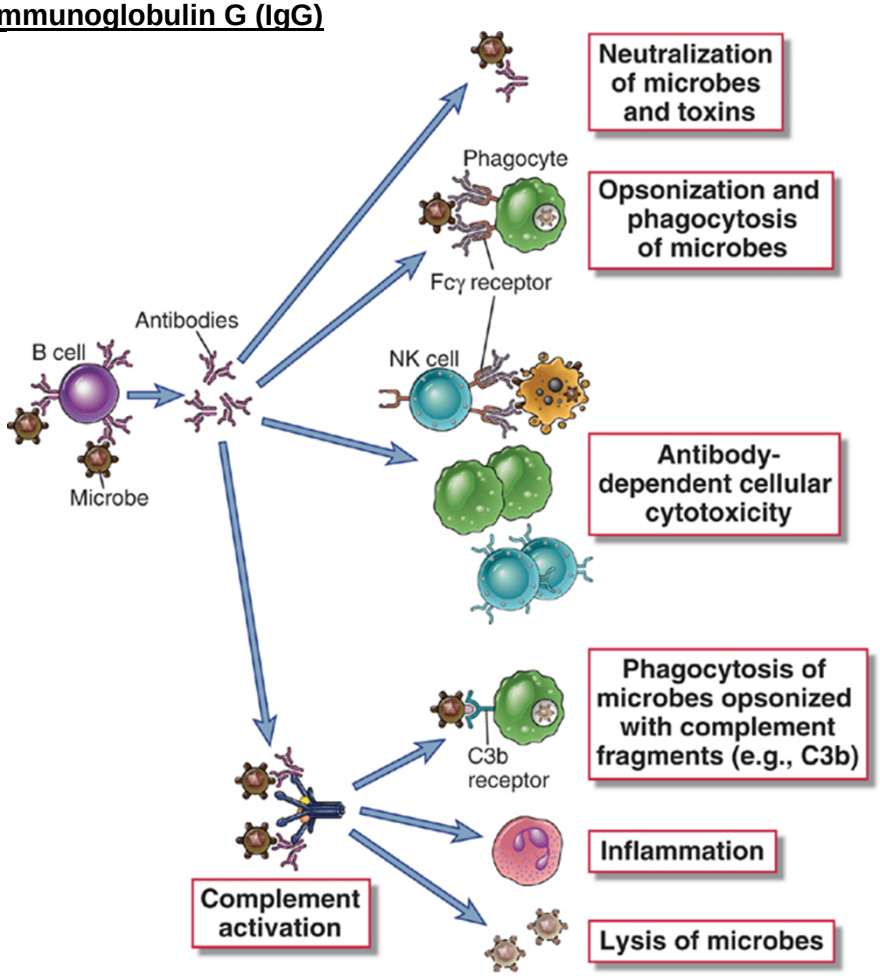
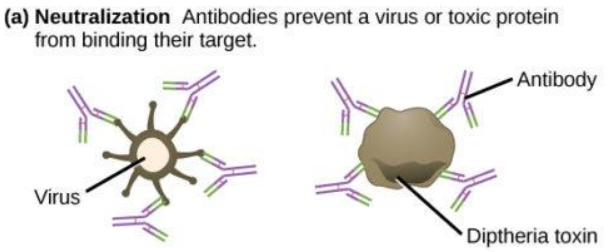
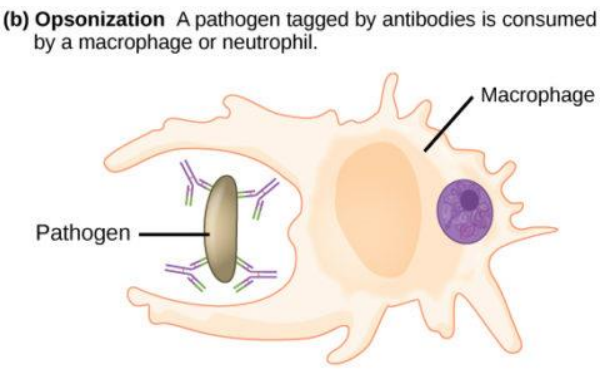


Fig. 3.1.4: Functions of IgG

- **Neutralisation of Pathogens or Toxins**
  - Binding of the antibody to the antigenic epitope on a pathogen or toxin **prevents it from attaching to the host cell receptor**.
- **Opsonisation**
  - Opsonisation refers to the process of **coating the surface of a pathogen to enhance phagocytosis**. After antibodies bind to a pathogen, the Fc portion of these antibodies bind to the Fc receptor of the phagocyte (neutrophils and macrophages) to promote phagocytosis.





- **Antibody-dependent cellular cytotoxicity (ADCC)**
  - Binding of IgG to target cell recruits natural killer cells to destroy the target cell via release of chemicals and proteins.
- **Complement activation**
  - The complement system consists of proteins and enzymes that sequentially activate each other to amplify the immune response.
  - Antibodies attached to the surface of a pathogen activates the complement system.
- **Agglutination of insoluble antigens**
  - Agglutination can result in immobilisation of motile bacteria, preventing their ability to spread or invade tissue

3.2 GENERATION OF ANTIBODY DIVERSITY

Learning Outcome (d)
Explain how somatic recombination, hyper-mutation and class switching result in millions of different antibody molecules.

- The immune system is capable of producing a wide repertoire of antibodies with specific antigen-binding sites. The following processes contribute to the diversity of antibodies:

  - Inheritance of a **large number of V, D, J gene segments** encoding immunoglobulin chains
    - A **heavy chain gene** contains **V** (variable) gene segments, **D** (diversity) gene segments, **J** (joining) gene segments and **C** (constant) gene segments.
    - A **light chain gene** contains **V** (variable) gene segments, **J** (joining) gene segments and **C** (constant) gene segments. Two types of light chains exist – **lambda (λ)** and **kappa (κ)**.
    - The multiple gene segments contribute to the vast number of heavy and light chain gene diversities.

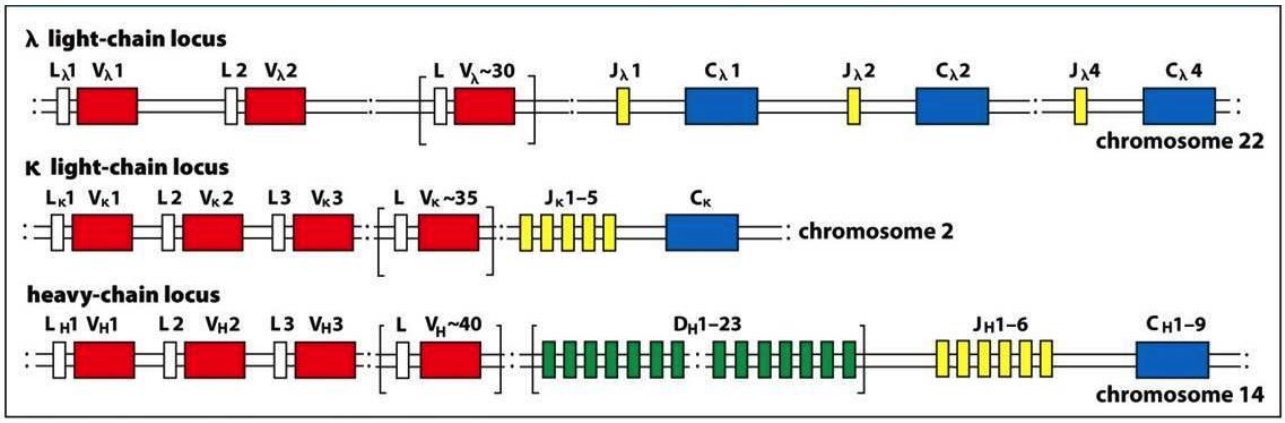
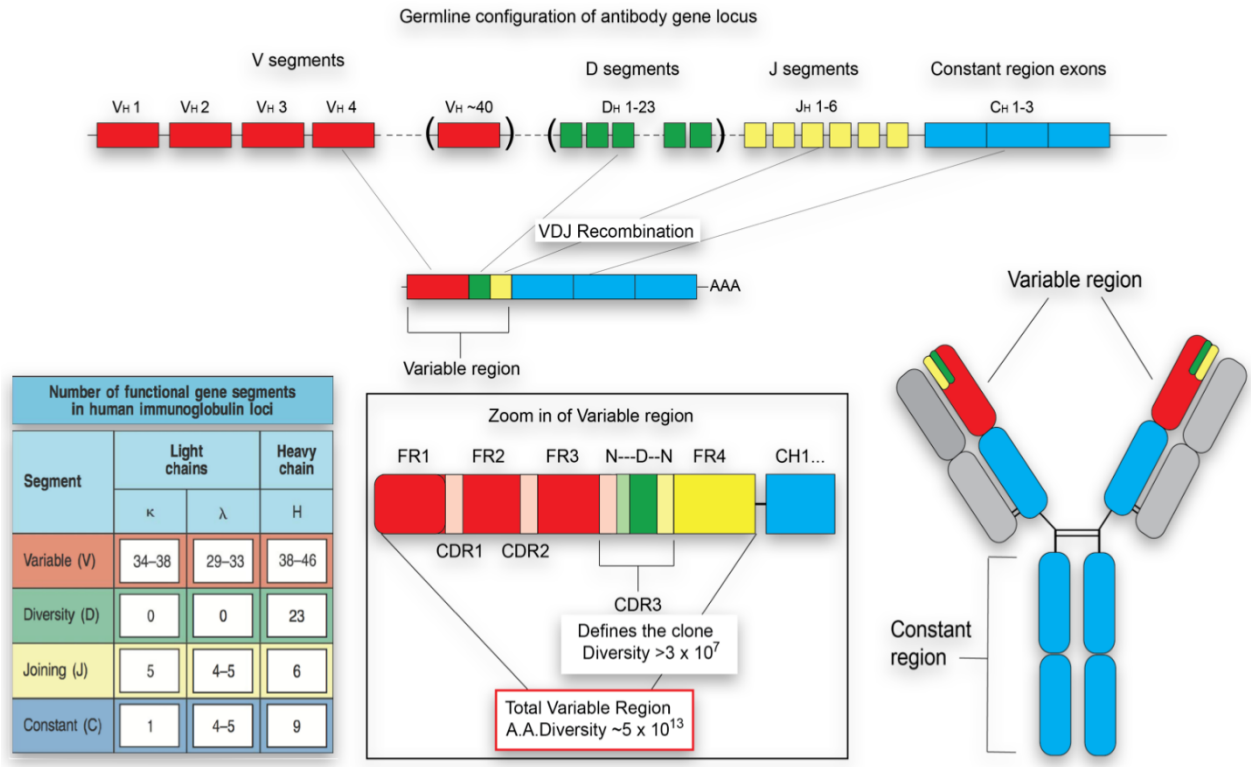


Fig. 3.2.1: Immunoglobulin (Ig) heavy and light chain gene loci

  - **Somatic Recombination** (or V(D)J recombination)
    - During B cell development in the bone marrow, **random rearrangement of gene segments** occur, where gene segments are cut and brought together by enzymes to form a single exon. This gives rise to **combinatorial diversity**, with a large number of possible combinations for the resultant **variable regions** for the heavy and light chains.

– At the heavy chain gene locus, any of **the V gene segments** can be joined to any of the **D gene segments** and any of the **J gene segments**.

– At the light chain gene locus, any of the **V gene segments** can be joined to any of the **J gene segments**.



Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	34–38	29–33	38–46
Diversity (D)	0	0	23
Joining (J)	5	4–5	6
Constant (C)	1	4–5	9

Fig. 3.2.2: Somatic recombination resulting in combinatorial diversification

– While most recombinations are site-specific, there exists slight variations in the positions joined, resulting in random loss or gain or nucleotides at joining sites. This gives rise to **junctional diversity**, resulting in **variations in amino acid sequences** at the **variable regions**, thereby increasing the diversity of antigen-binding sites.

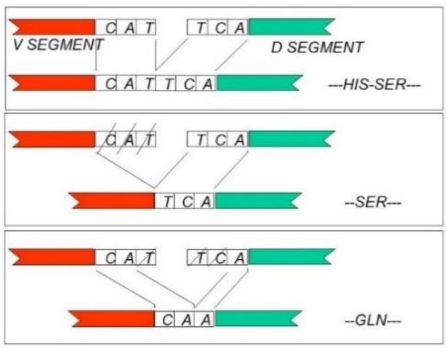


Fig. 3.2.3: Somatic recombination resulting in junctional diversity

– After somatic recombination, transcription occurs to produce the pre-mRNA containing the VDJ exon (for heavy chain) / VJ exon (for light chain) and the C gene segment. The pre-mRNA undergoes splicing, to join the VDJ / VJ exon with the C segment. The mature mRNA is then translated to form the heavy chain and light chain of the immunoglobulin respectively. This results in **expression of IgM** on the cell surface membrane of a **naïve B cell**.

– **Affinity Maturation**
  – After a naïve B cell has encountered an antigen, the activated B cells downregulate expression of membrane Ig and undergo clonal expansion. Some of these B cells can undergo **somatic hypermutation**.

  – Somatic hypermutation is a **random point mutation** in the **rearranged VDJ region** of the heavy chain locus or **rearranged VJ region** of the light chain gene locus. This results in a change in the **nucleotide** sequence, which results in a change in the **amino acid sequence** of the **antigen-binding site** of the **variable regions (V_H and V_L)**. This causes a change in the **3D conformation** of the antigen-binding site, thereby increasing the diversity of antibodies.

  – These hypermutations are random and can either increase or decrease affinity of the antibody for the antigen, or can have no effect on the affinity.

  – After the hypermutation, the B cells re-express their membrane Ig. Only B cells expressing **antibodies with high affinity** will be selected to undergo **clonal expansion** and **differentiate into plasma cells and memory B cells**. B cells with low affinity for antigen will undergo apoptosis.

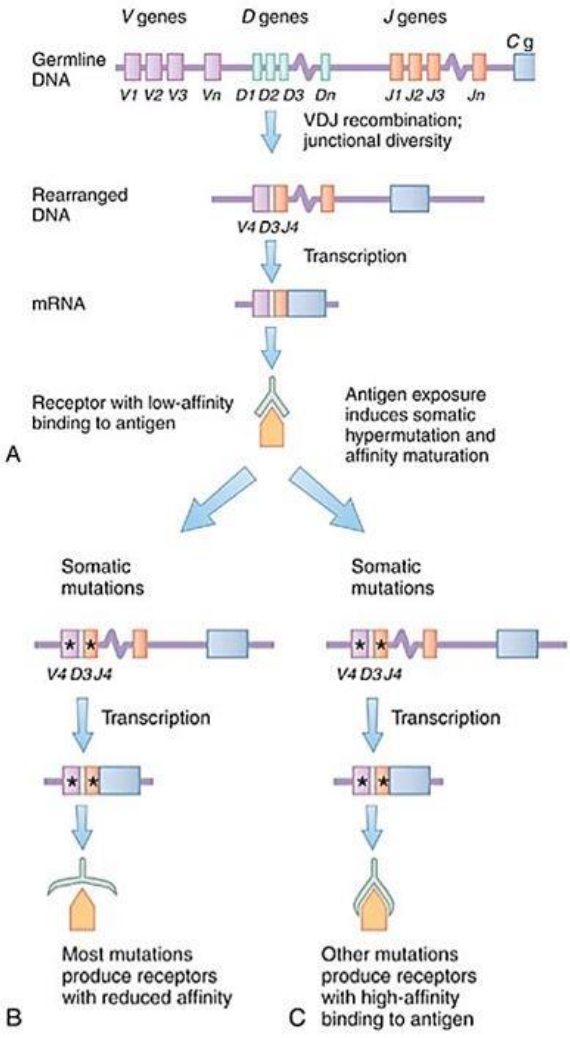


Fig. 3.2.4: Affinity maturation

– **Class Switching**
  – During class switching, the **variable regions of both the heavy and light chain (V_H and V_L) is retained** while the **constant regions of the heavy chain (C_H) is changed**, producing a **different class of antibody** with the **same antigen specificity**.

  – Class switch is controlled by **cytokines** released from helper T cells.

  – This results in **DNA rearrangement** at the **C (constant) gene segment of the Ig heavy chain gene locus**. The V_H DNA is moved from being immediately upstream of the C_μ gene segment to being upstream of another C gene segment (e.g. C_γ). This results in a class switch from IgM to another class (e.g. IgG).

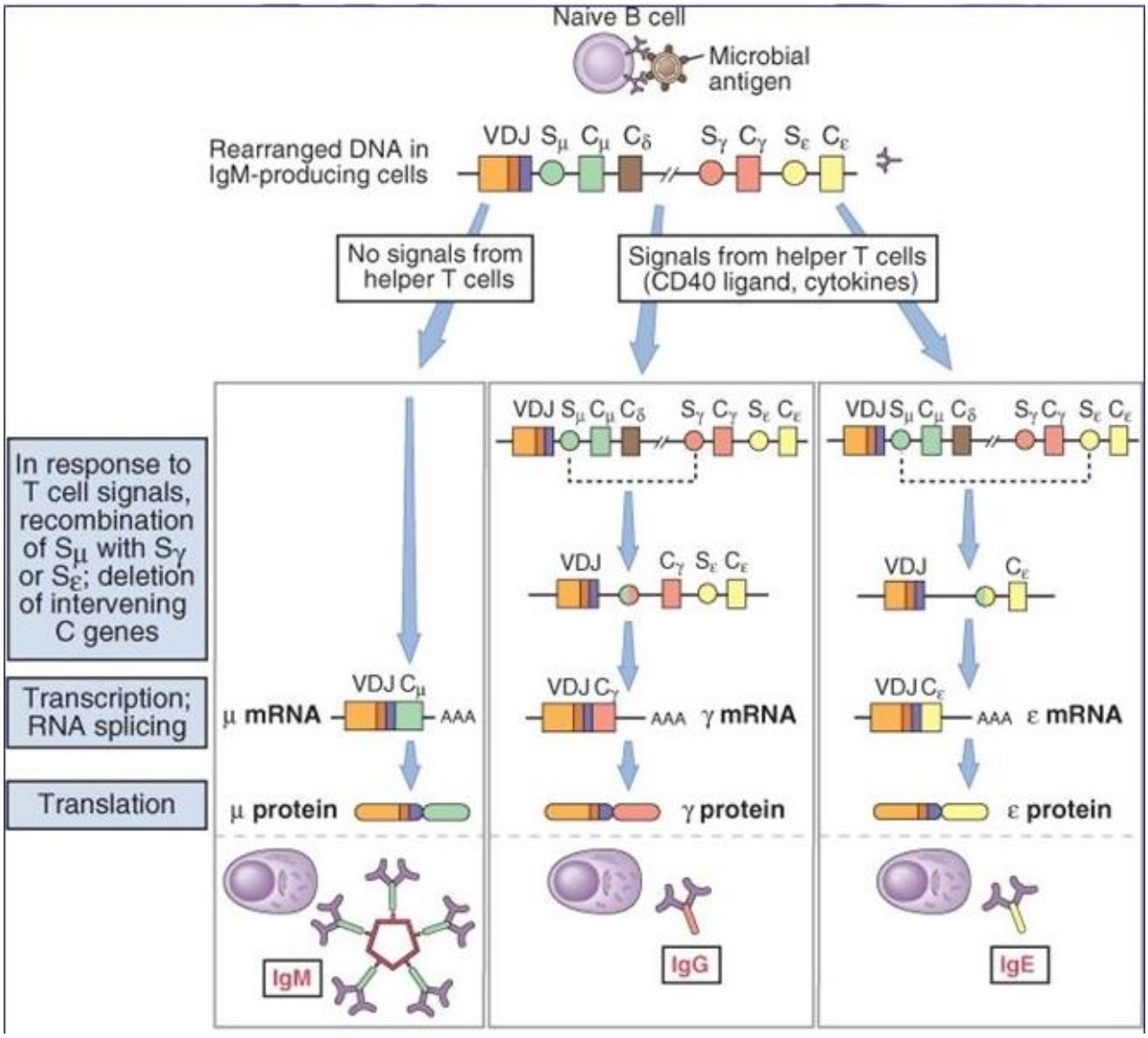


Fig. 3.2.5: Class switching

4 IMMUNOLOGICAL MEMORY

Learning Outcome (b)
Outline the roles of B lymphocytes, T lymphocytes, antigen-presenting cells and memory cells in specific primary and secondary immune responses

- The adaptive immune response results in activation of lymphocytes to remove pathogens from the host, as well as establishment of a population of lymphocytes, i.e. **memory cells**, to protect against reinfection.

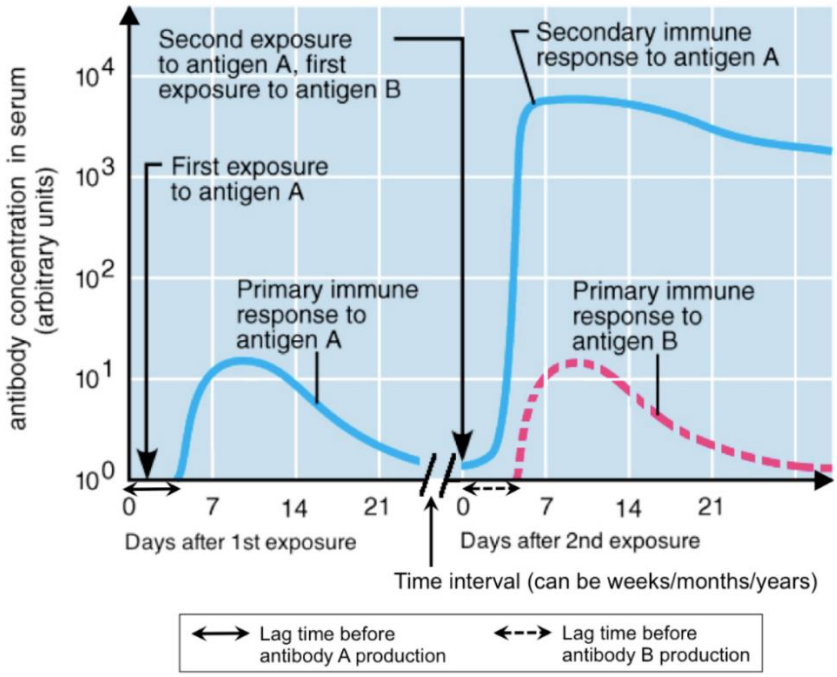


Fig. 4.1: Antibody concentration during primary and secondary immune response

- On the **first exposure** of an individual to a foreign antigen, the **primary immune response** occurs.
  - Presence of lag period (~3 to 6 days) between encountering antigen and production of antibody. This reflects the time required for clonal expansion and differentiation of naïve B and T cells to plasma cells and effector T cells respectively.
  - The antibody concentration rises gradually, with a lower peak concentration. This shows that the strength of the primary response is weaker than the secondary response.
  - The antibody concentration decreases after a short period, which shows that the duration of the response is shorter than the secondary response.
- The primary response results in production of memory B and T cells, resulting in **immunological memory**.

- On the second exposure of an individual to the **same** antigen, the **secondary immune response** occurs to produce a **faster and stronger immune response**.

  – Memory B and T cells can be reactivated easily to divide and differentiate to form effector cells. As memory B cells have already undergone affinity maturation and class switching, this results in **rapid production** of **high affinity antibody**.

  – Memory B cells are able to respond to low doses of antigen, hence they can be activated easily. Memory B cells have also already undergone proliferation, resulting in more antigen-specific memory B cells that can respond to the antigen.

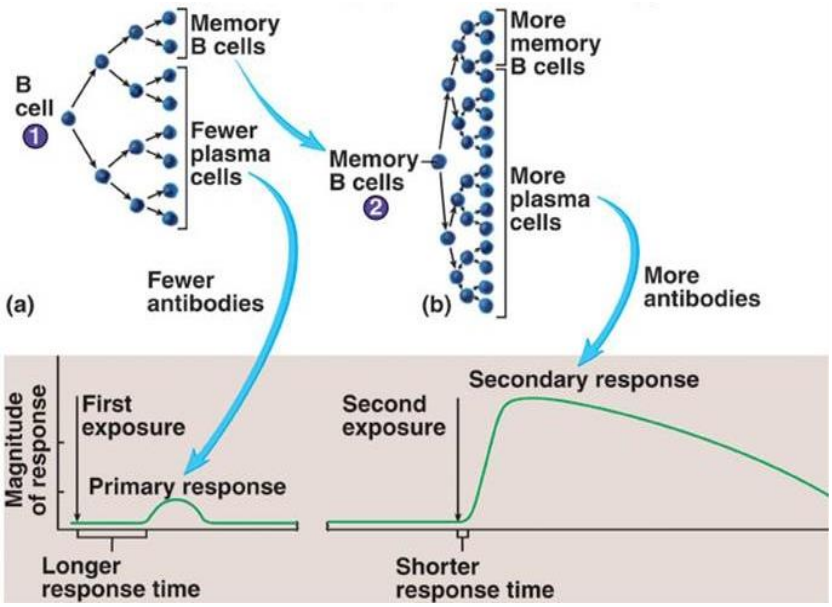


Fig. 4.2: Antibody concentration during primary and secondary immune response (2)

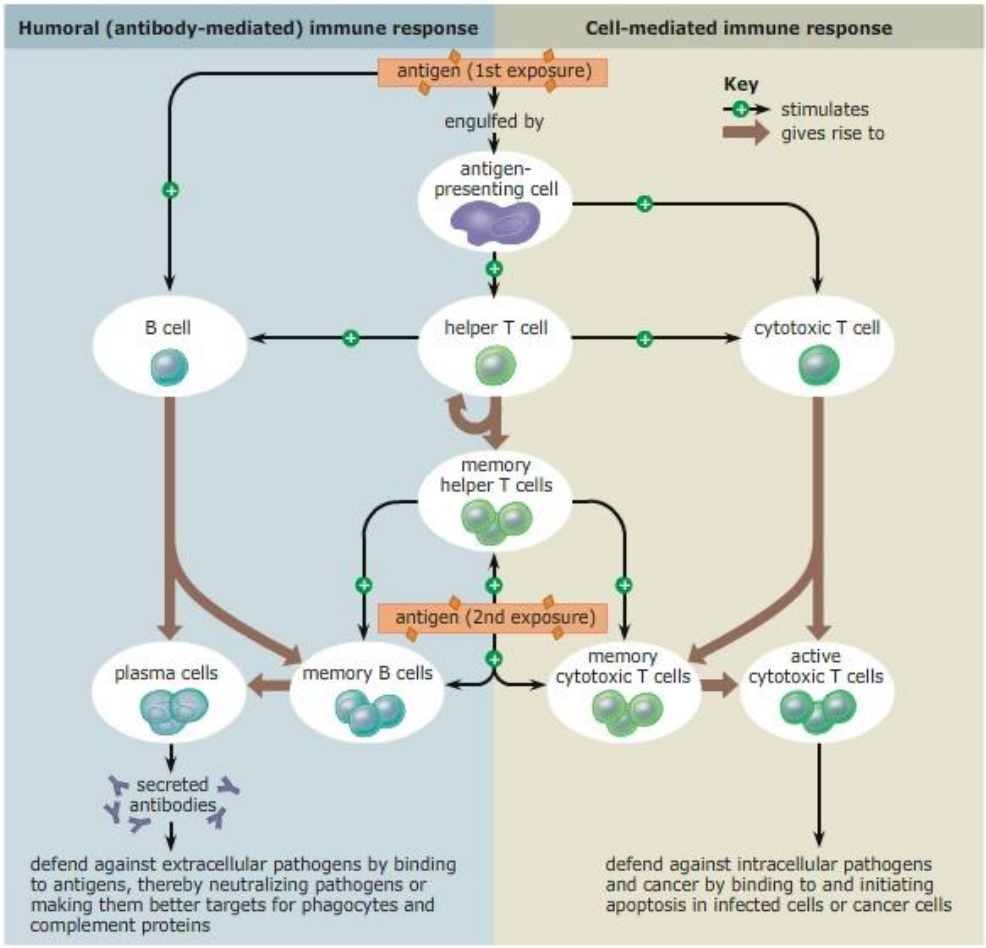


Fig. 4.3: Primary and secondary immune response

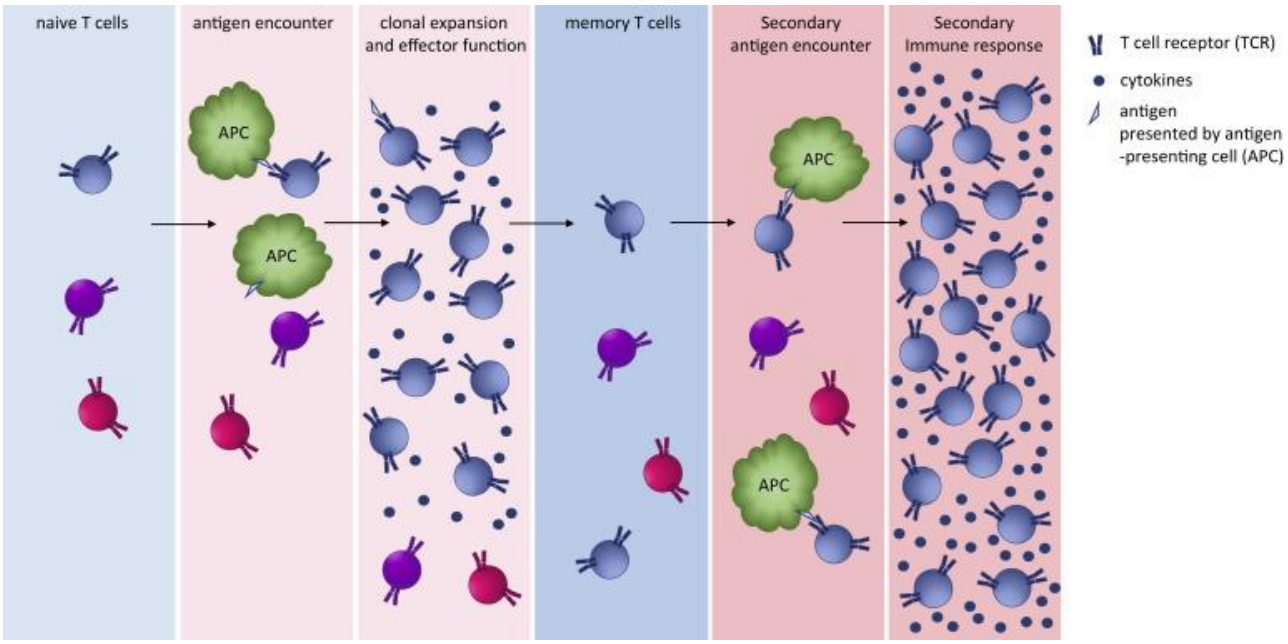


Fig. 4.4: T cell activation during primary and secondary response

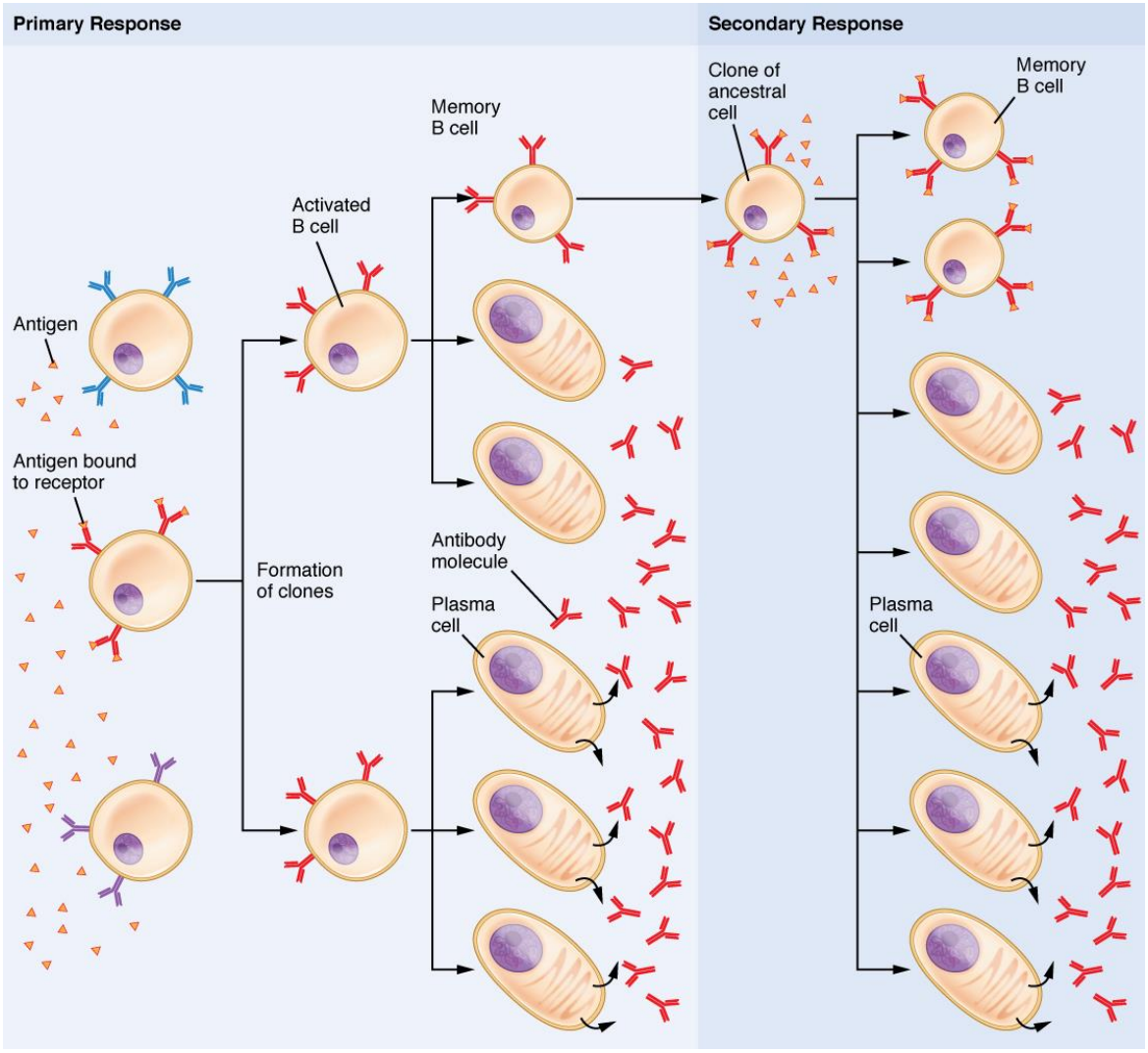


Fig. 4.5: B cell activation during primary and secondary response

5 HUMAN HEALTH AND DISEASES

5.1 VIRAL DISEASES

Learning Outcome (g)
Explain how viruses, including influenza virus and HIV, cause diseases in humans through the disruption of host tissue and functions

- Viruses generally produce diseases in their hosts through various routes:
  (a) **Disrupting normal cellular activities** e.g. DNA, RNA and protein synthesis which results in functional defects in cells which will then trigger programmed cell death.
  (b) **Deplete essential cellular materials** such as amino acids, nucleotides, ATP which is necessary for normal functioning of cells.
  (c) Alter the host cell membrane by **inserting viral glycoproteins** that result in the cell being recognized as foreign by immune cells and being destroyed in the process.
  (d) Cause **cell lysis** which will kill cells. Cell lysis is triggered by the release of hydrolytic enzymes from lysosomes. When enough cells and tissues are destroyed, there would be a loss of physiological function for the affected tissue.
  (e) Cause the infected cells to produce **toxic substances** that lead to disease symptoms. E.g. Japanese encephalitis viruses which infect immune cells in the central nervous system cause it to produce cytokines which cause toxic effect in the brain.
  (f) Have **molecular components that are toxic**, such as envelope proteins. E.g. The HIV envelope protein, gp120, is toxic to neurones, induces abnormal development of nerve cells.
  (g) **Suppress the immune mechanism** of the host and **increase susceptibility to secondary infections**. E.g. HIV
  (h) **Cause canc**er in the host. Though the mechanism is unknown, it is proven that some viral infections cause the host cells to divide uncontrollably, forming tumours. Viruses capable of inducing tumors in animals are called oncogenic viruses.

  Both DNA- and RNA-containing viruses are capable of inducing tumors. Some examples of viruses that cause cancer are Human T-cell leukemia virus (type 1), Epstein-Barr virus, Hepatitis B virus and Papilloma virus.

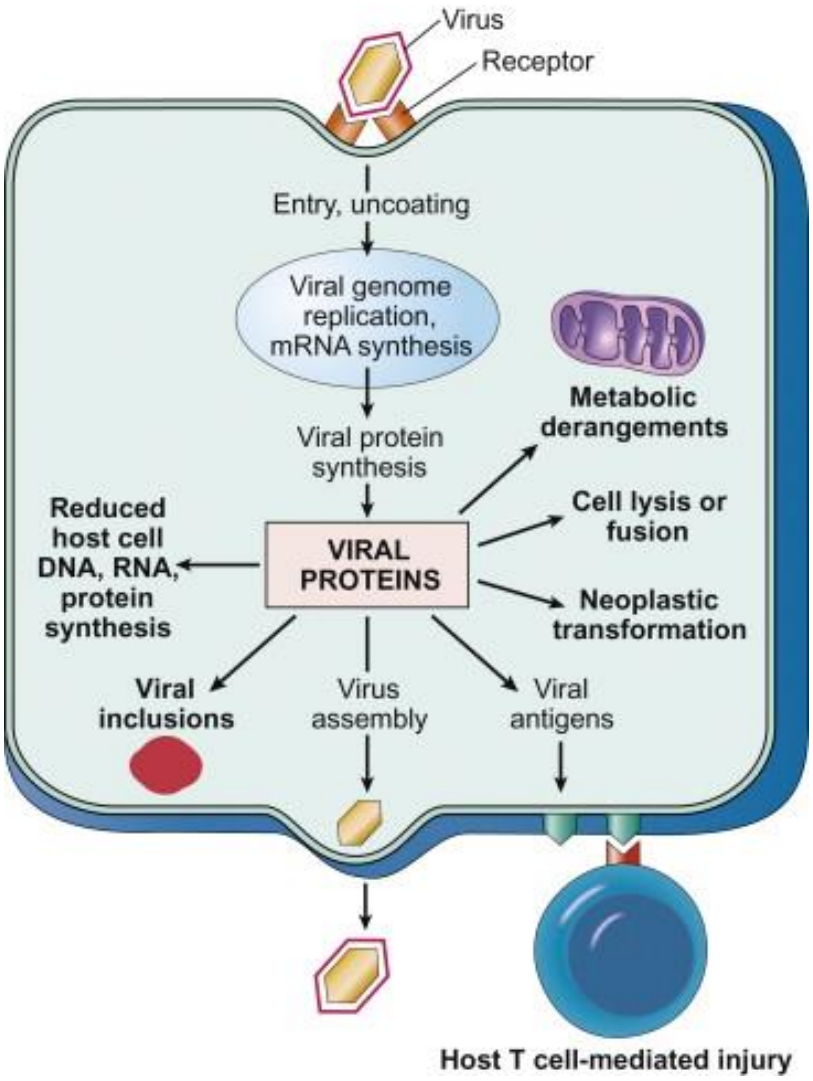


Fig. 5.1.1: Pathogenesis of viral infections and diseases

5.1.1 How Influenza Infections Cause Disease

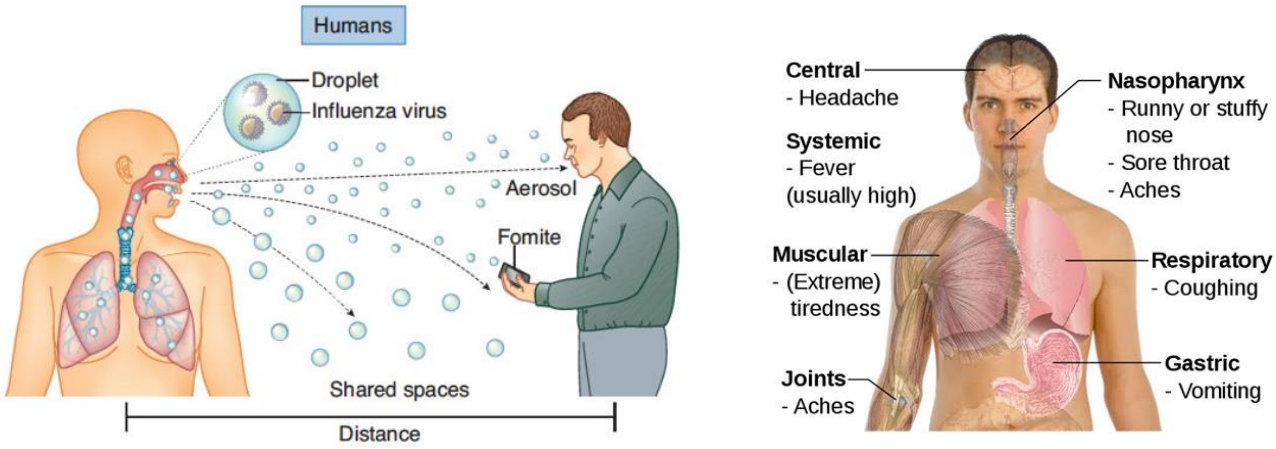


Fig. 5.1.1.1: Mode of transmission of influenza virus (left) and symptoms of influenza infection (right)

Clinical Features

- Incubation period: ~2-3 days
- Signs and Symptoms: Shivering, discomfort, headache, aching in the limbs and back, high fever. Sore throat and runny nose may accompany fever.

Pathogenesis

- Infection is spread via **respiratory droplets**. Influenza virus targets mainly the epithelial cells of the respiratory tract.
- The virus takes over cellular machinery and resources of the epithelial cells for replication. This disrupts normal cellular activities, and depletes essential cellular materials, eventually causing **cell death**.
- Viral glycoproteins embedded in infected epithelial cell membrane may cause immune system to recognize cells as foreign and be destroyed by the immune system.
- Viral replication in epithelial cells results in the following:
  - In the **nose and sinus passages**: Destruction of the cilia, resulting in a decrease in efficiency of mucus removal in the airway.
  - In the **lower respiratory tract** (e.g. alveoli): Massive discharge of fluid into the air sacs, resulting in breathing difficulties, as well as lowering of resistance to secondary invaders (e.g. bacteria), which may result in pneumonia and respiratory failure.

5.1.2 How HIV Infections Cause Disease

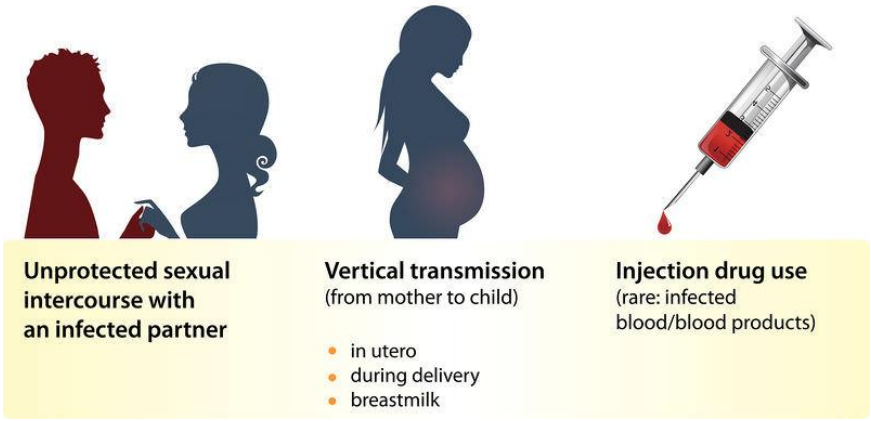


Fig. 5.1.2.1: Mode of transmission of HIV

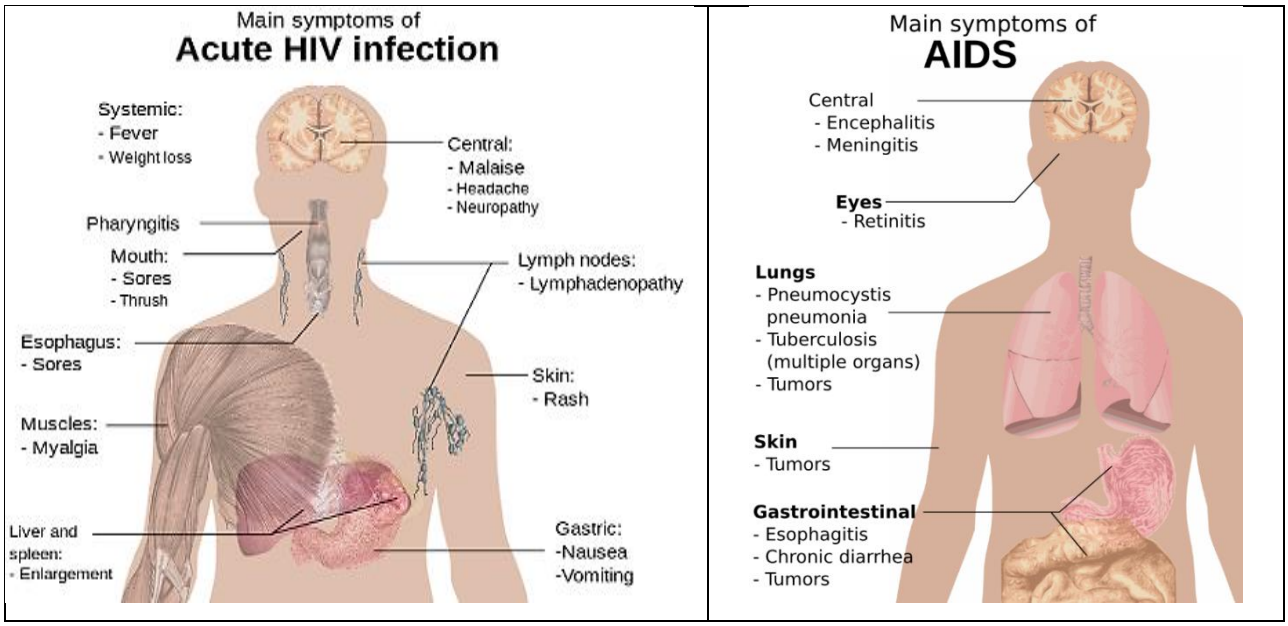


Fig. 5.1.2.2: Symptoms of HIV infection and AIDS

Clinical Features

- Incubation period: ~2-6 weeks where the infection is asymptomatic (no symptoms are observed)
- Signs and Symptoms:
  - **Acute** phase: Develop flu-like symptoms such as fever, lethargy, nausea, diarrhoea, headaches, stiff neck, swelling of lymph nodes.
  - **Latent** infection: This stage lasts from 2 weeks to 20 years. Majority of individuals are **asymptomatic** as the HIV remains latent within the host cell as a provirus.
  - Full-blown AIDS: Clinical manifestations summarized as malignant (cancer e.g. Kaposi's sarcoma, B cell lymphoma), infective (infections of skin/ mucous membranes e.g. chronic sinusitis) and neurological (e.g. dementia, motor dysfunction).

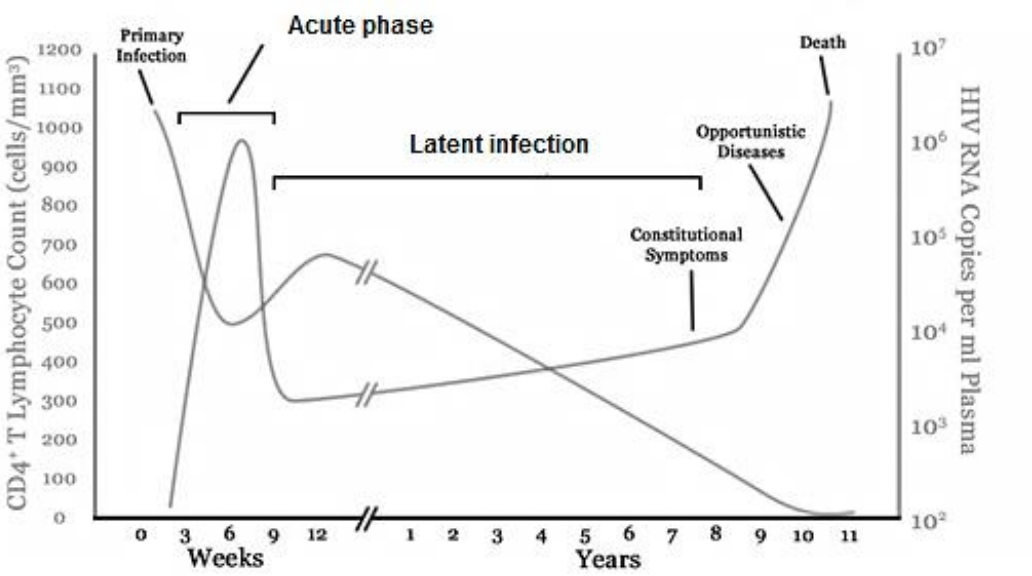


Fig. 5.1.2.3: Graph of CD4+ T lymphocyte count and HIV RNA copies upon HIV infection

Pathogenesis

- Infection is spread mainly through unprotected sex with an infected partner, or sharing of needles with an infected person.
- HIV infects **CD4 helper T cells and macrophages** of the immune system. A decrease in levels of helper T cells and macrophages due to viral infection will severely impair the immune system.
- In the **acute phase**, the virus enters the bloodstream and targets cells in the immune system. High levels of virus are found circulating in the blood and lymphatic system, but following a vigorous immune response, a sharp decline in viral levels results.
- During **latent infection**, the infected individual may be **asymptomatic** but **HIV remains latent in host cell as a provirus**. Hence, viral replication continues within the lymph nodes and there is a **progressive loss of CD4 cells**.

  The loss of CD4 cells eventually lead to a depression in the immune system known as **Acquired Immune Deficiency Syndrome (AIDS)**.
- When the immune system of the infected patient is crippled, it results in an **increased susceptibility to opportunistic infections** (an infection by a microorganism that normally does not cause disease but becomes pathogenic when the body's immune system is impaired and unable to fight off the infection).

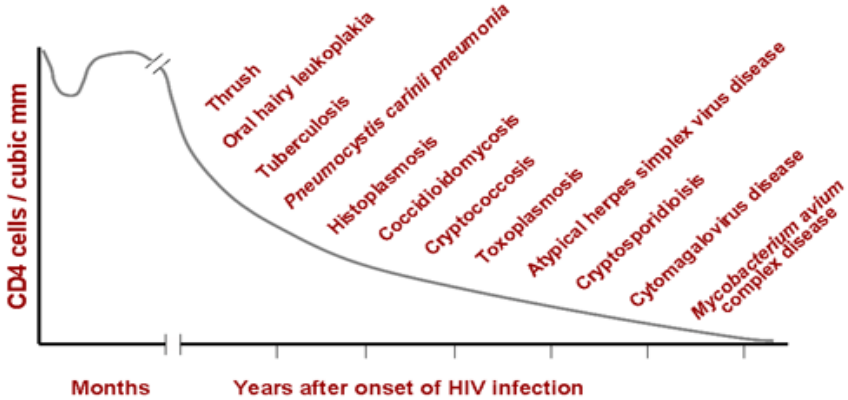


Fig. 5.1.2.4: Oppotunistic infections caused by HIV infection

5.2 BACTERIAL DISEASES (TUBERCULOSIS)

Learning Outcome (h)
Explain the mode of transmission and infection of bacterial pathogens, using *Mycobacterium tuberculosis* as an example

- Tuberculosis (TB) is an **airborne infectious disease** caused by *Mycobacterium tuberculosis.*
- Bacteria are transmitted from person to person via **fine, aerosol droplets** released when the infected person **coughs or sneezes** and an uninfected person inhales these droplets.
- *M. tuberculosis* usually affects the lungs (pulmonary tuberculosis), but it can cause disease in almost any part of the body such as the brain, lymph nodes, the kidneys, bones, and joints (extrapulmonary tuberculosis).
- Symptoms of TB include a persistent cough that lasts three weeks or longer, low-grade fever, night sweats, fatigue, weight loss, chest pain, coughing up blood or sputum

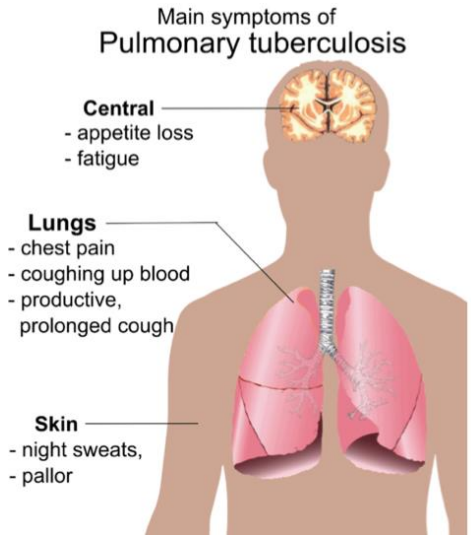


Fig. 5.2.1: Symptoms of TB infection

Mode of Infection

- *M. tuberculosis* infection occurs when bacteria dispersed in the air from a patient with active pulmonary TB reach the alveoli of the host. Alveolar **macrophages** phagocytose the bacteria and form phagosomes containing *M. tuberculosis.*
- Inside the phagosome, *M. tuberculosis* inhibits the fusion of the phagosome with lysosomes. This **prevents formation of phagolysosome**, hence lysosomal enzymes are not available to kill the bacteria. *M. tuberculosis* survives and continues to multiply inside the macrophages.
- This induces release of cytokines that initiates the inflammatory response in the lungs, causing an accumulation of neutrophils and macrophages at the site of infection, forming a **granuloma**.

  The function of the granuloma is to segregate the infection to **prevent spread** to the remainder of the lung and to other organs, as well as to **concentrate the immune response** directly at the site of infection.
- The granuloma is maintained in a persistently infected host, allowing the bacteria to persist in a dormant stage, resulting in **latent tuberculosis**.
- Most individuals who develop tuberculosis (usually after immunosuppression of the host due to genetic or environmental factors) do so after a long period of latency where the **granuloma breaks down to release the bacteria**.

The bacteria then travel to other sites within the lung and through the bloodstream to other organs to result in inflammation and massive tissue death leading to death of the untreated, infected person.

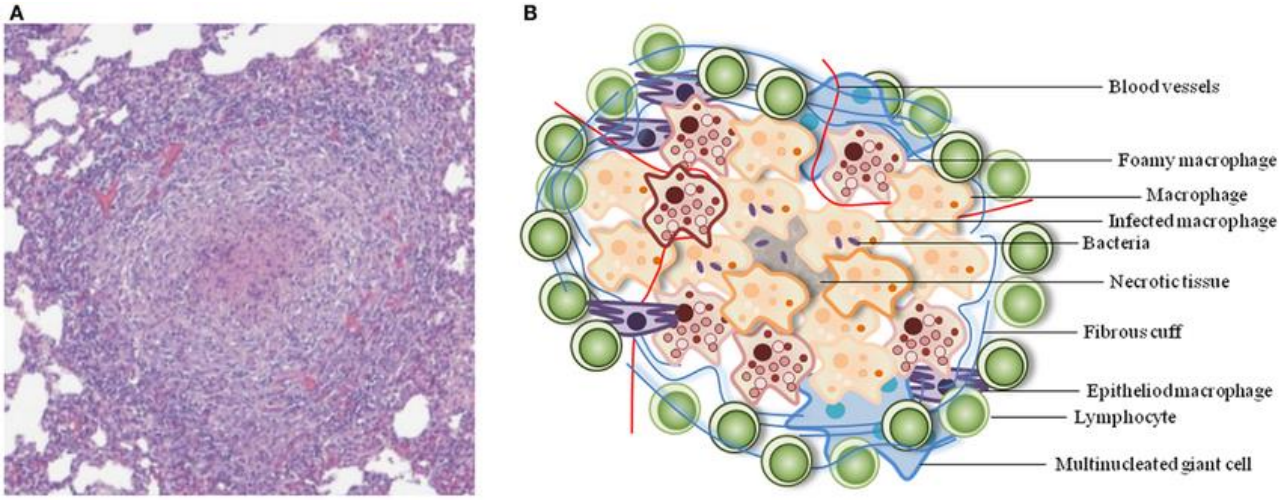


Fig. 5.2.2: Structure of a granuloma

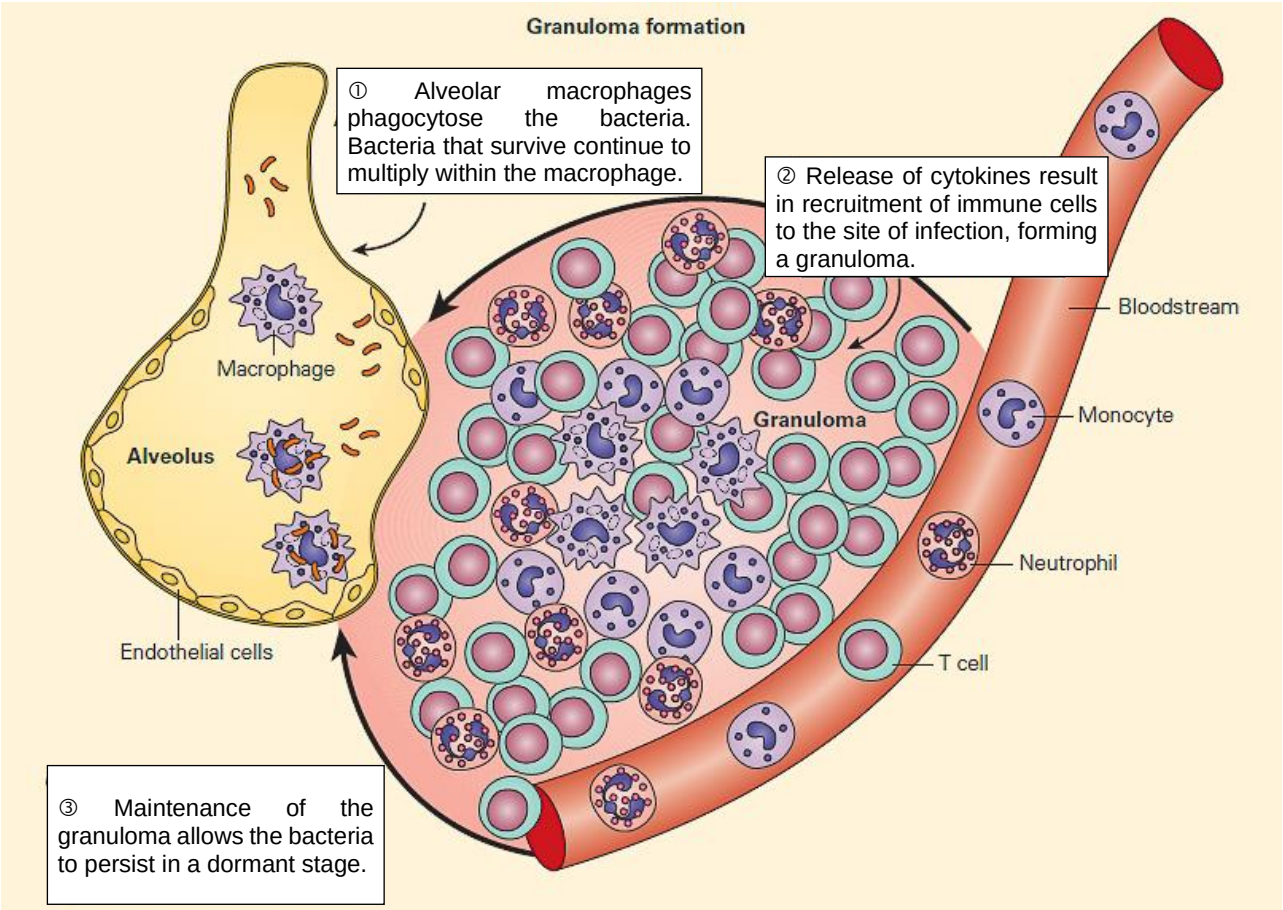


Fig. 5.2.3: Granuloma formation

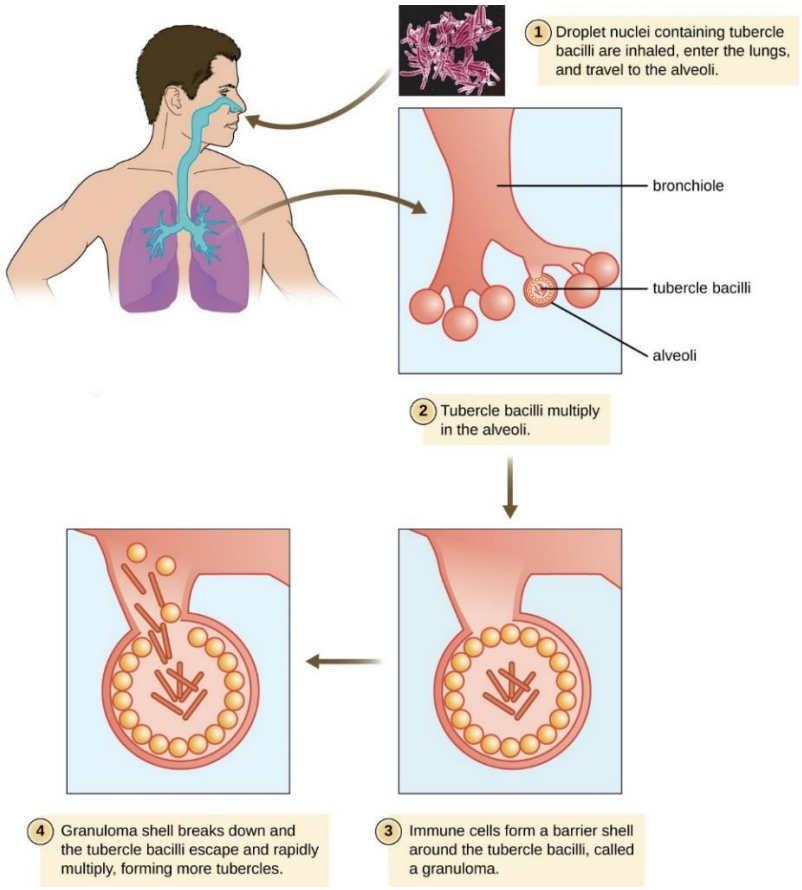


Fig. 5.3.2: Mode of infection of Tuberculosis mycobacterium(1)

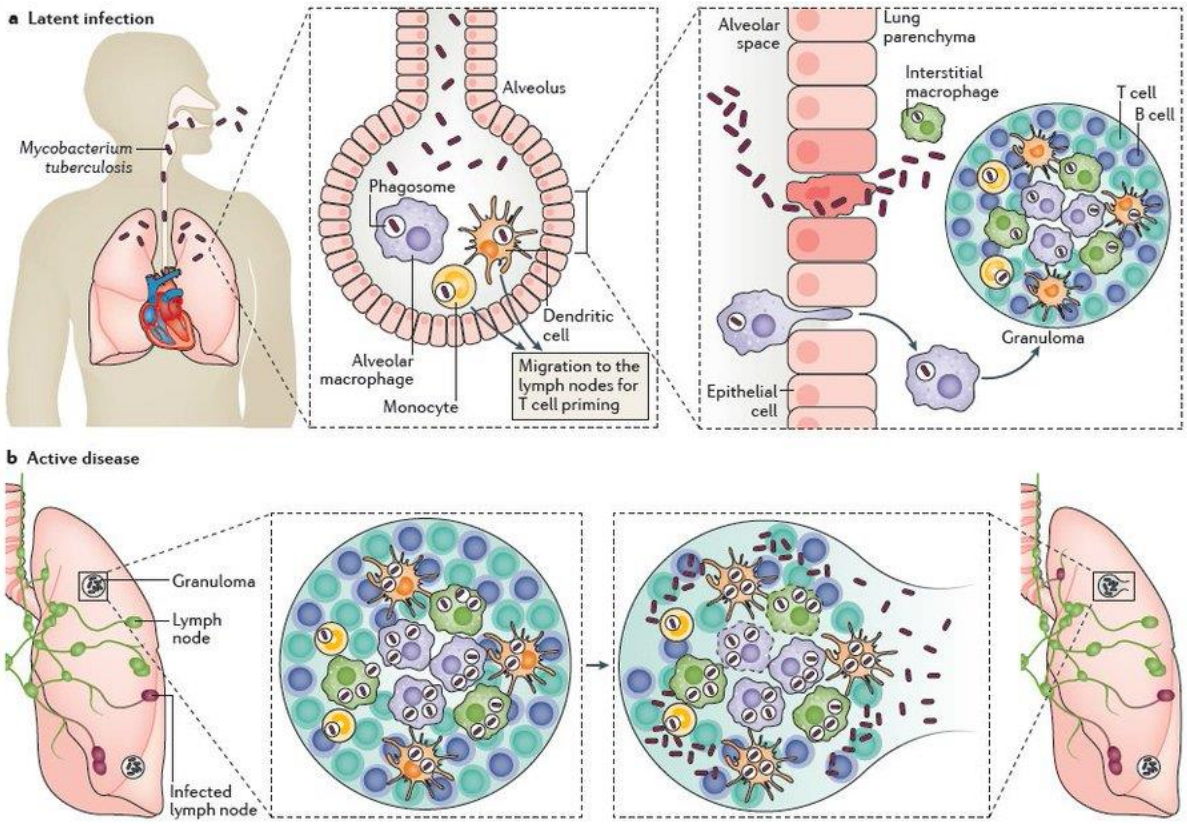


Fig. 5.3.2: Mode of infection of Tuberculosis mycobacterium (2)

6 MEDICAL INTERVENTIONS

6.1 ANTIBIOTICS

Learning Outcome (i)
Describe the modes of action of antibiotics, including penicillin, on bacteria.

- Antibiotics inhibit the growth of bacteria or kill the bacteria by disrupting the metabolism of the prokaryotic cells.
  (i) A **bacteriostatic antibiotic** is one that is able to **inhibit the growth or cell division** of bacteria.
  (ii) A **bactericidal antibiotic** is one that is able to **kill bacteria** when the bacteria are in the process of undergoing cell division by binary fission.
- Broad-spectrum antibiotics are effective against many types of bacteria, while narrow-spectrum antibiotics are effective against just a types of bacteria.
- Antibiotics can be classified into different categories based on their mechanism of action. Antibiotics disrupt prokaryotic metabolic pathways such as:
  (i) Protein synthesis (Translation)
  (ii) Nucleic acid synthesis
  (iii) Cell wall synthesis

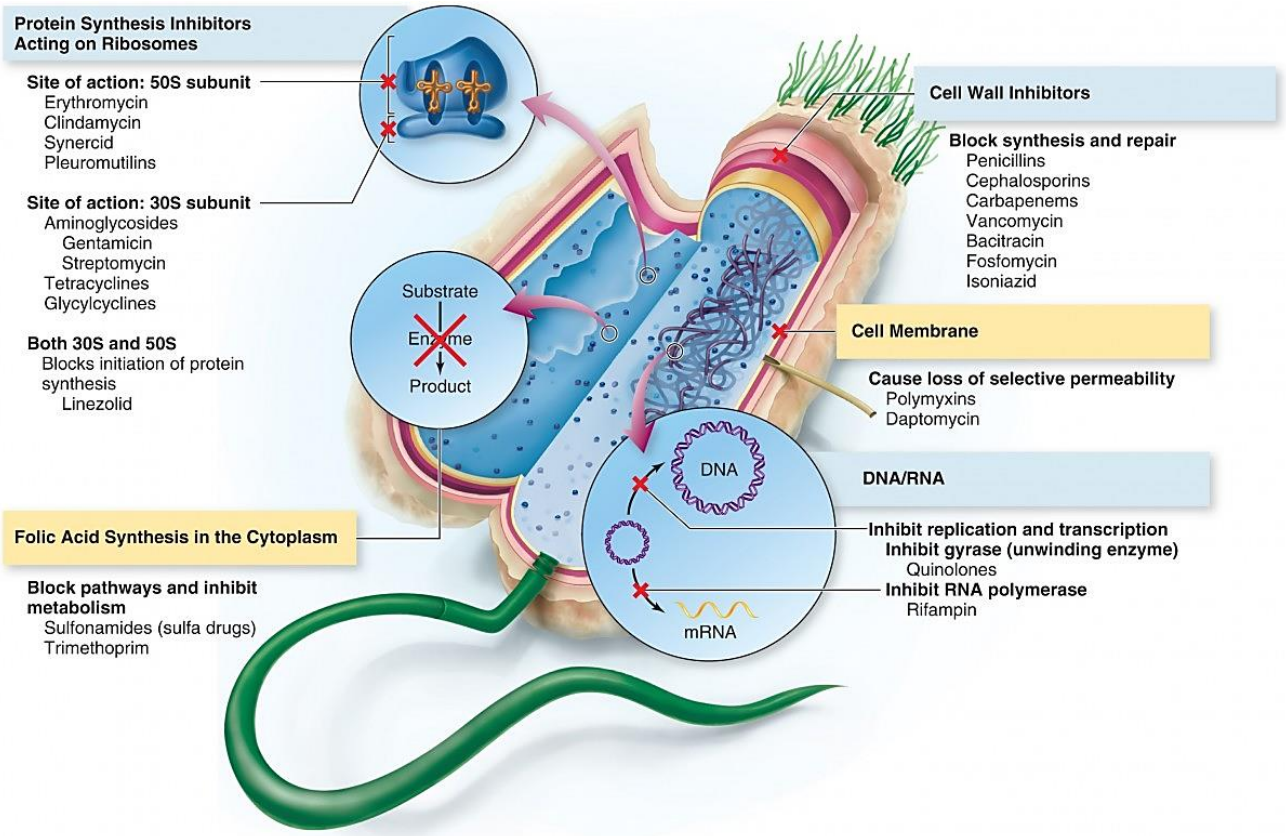


Fig. 6.1.1: Mode of action of antibiotics

6.1.1 Inhibition of Protein Synthesis

- Enzymes and many important biomolecules in cells are proteins in nature. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells.
- Therefore, many types of antibacterial drugs target bacterial protein synthesis by binding to either the **30S or 50S subunits of ribosomes**. This prevents protein synthesis in bacteria, inhibiting their growth and multiplication which might consequently lead to bacterial death.
- Examples: aminoglycosides, lincosamides, streptogramins, chloramphenicol, tetracyclines
  - Streptomycin binds to the small subunit (30S) of the bacterial ribosome, such that the initiator tRNA cannot bind to the small subunit. This prevents the formation of a complete translation initiation complex, hence inhibiting initiation of translation.
  - Tetracycline blocks aminoacyl-tRNA from attaching to the A site of bacterial ribosome. This prevents translation elongation from proceeding, even though translation initiation might have occured.

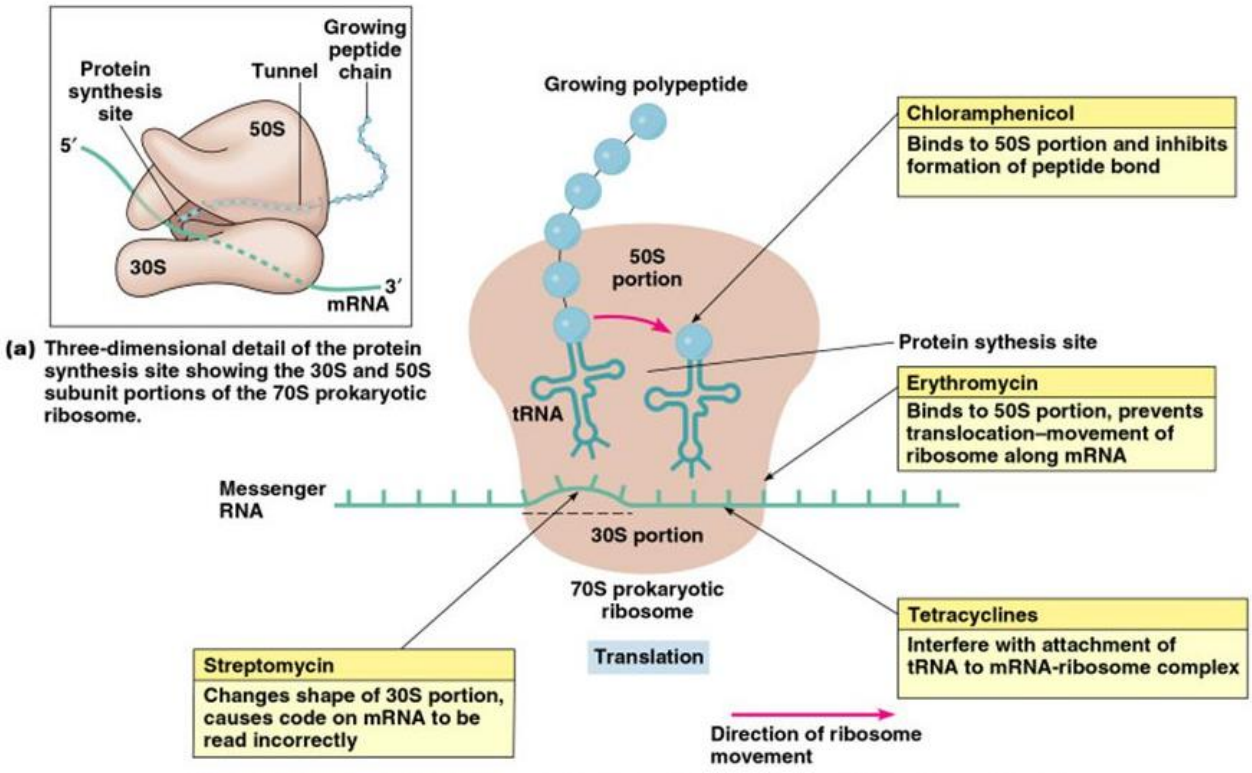


Fig. 6.1.1.1: Mode of action of antibiotics that inhibit protein synthesis

6.1.2 Inhibition of Nucleic Acid Synthesis

- DNA and RNA are important biomolecules, which functions as genetic material in all living organisms, including bacteria.
- Some antibiotics work by binding to components involved in DNA replication or RNA synthesis, disrupting these processes, thereby preventing bacterial multiplication and survival.
- Examples: quinolones, metronidazole, rifampin
  - Rifampin inhibits RNA synthesis by binding to bacterial RNA polymerase, thus preventing transcription.

6.1.3 Inhibition of Cell Wall Synthesis (by Penicillin)

- **Peptidoglycan** is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of most bacteria, forming the cell wall. Its main function is to preserve cell integrity and shape.
- Peptidoglycan is composed of chains of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues, which are covalently crosslinked via short peptides.
- During bacterial growth and division, peptidoglycan is continuously synthesized and remodeled via the action of specific enzymes called **transpeptidases**.

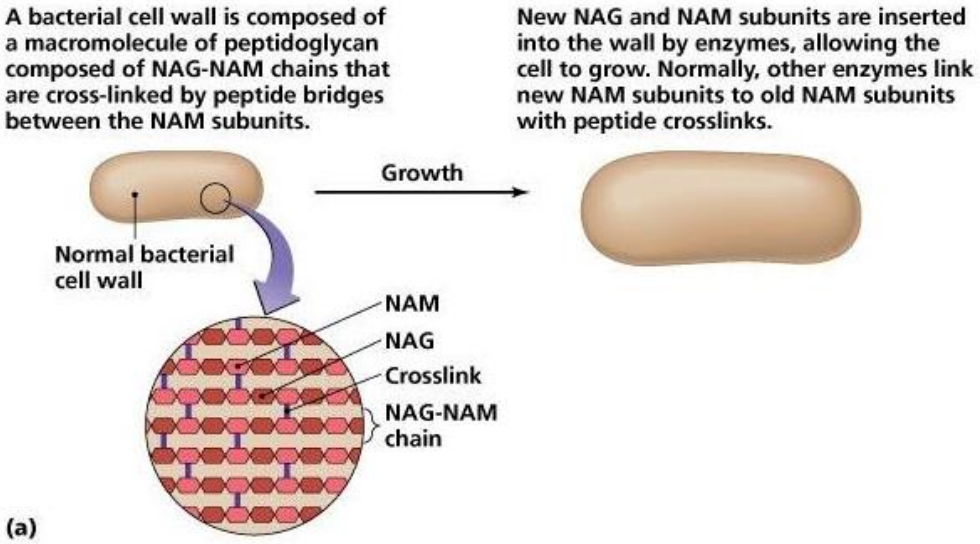


Fig. 6.1.3.1: Peptidoglycan cell wall synthesis

- **Pencillin** is produced by the fungus *Penicillium notatum*. It contains a four-membered **beta-lactam ring**.

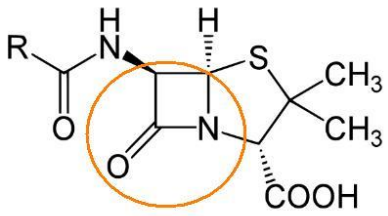


Fig. 6.1.3.2: Structure of penicillin with β-lactum ring circled

Mode of Action of Penicillin

- Penicillin binds irreversibly to the active site of **transpeptidase**, preventing the enzyme from cross-linking the peptidoglycan strands.

- This results in **weak cell walls** synthesized without intact peptidoglycan cross-links. These cell walls are prone to collapse and disintegrate when the bacteria attempts to divide.

- When bacteria take in water by osmosis, the increased turgor pressure against the weakened cell wall causes the bacteria to swell and lyse (i.e. **osmotic lysis**).

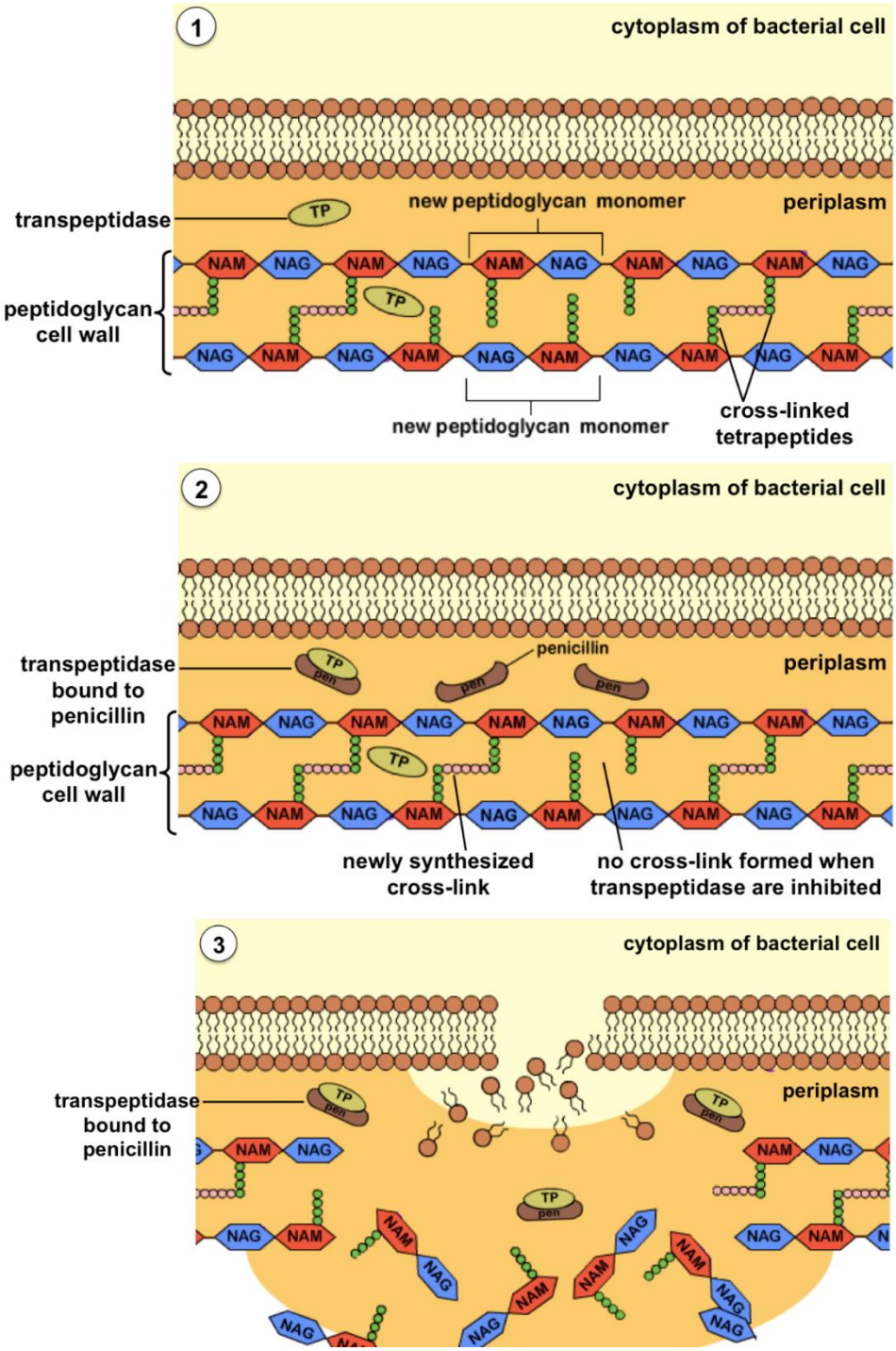


Fig. 6.1.3.3: Mechanism of action of penicillin

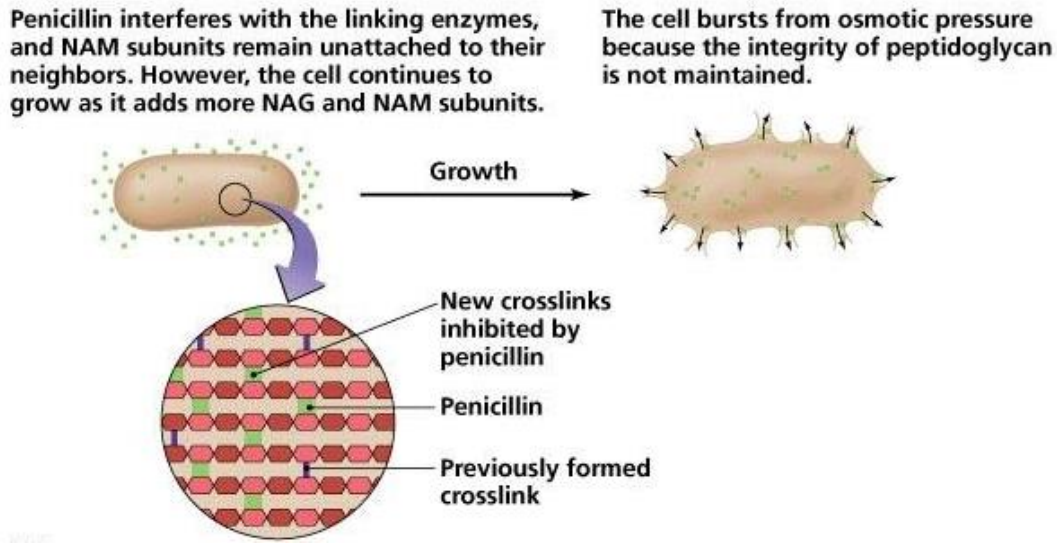
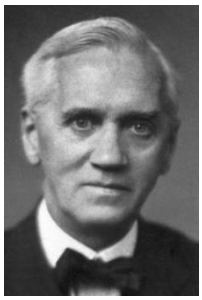


Fig. 6.1.3.3: Mechanism of action of penicillin (2)

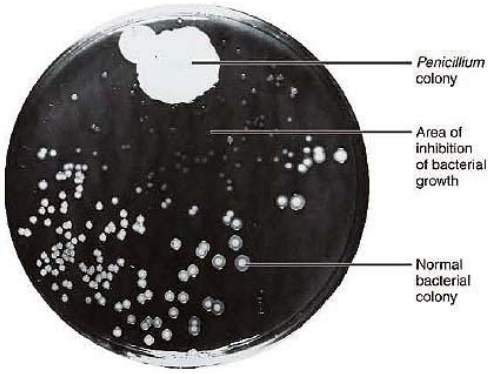
DID YOU KNOW?

Discovery of Penicillin



Alexander Fleming (1881-1955) was a Scottish physician-scientist who was recognised for discovering penicillin.

Returning from holiday on September 3, 1928, Fleming began to sort through petri dishes containing colonies of *Staphylococcus*, bacteria that cause boils, sore throats and abscesses. He noticed something unusual on one dish. It was dotted with colonies, save for one area where a blob of mold was growing. The zone immediately around the mold—later identified as a rare strain of *Penicillium notatum*—was clear, as if the mold had secreted something that inhibited bacterial growth.



The discovery of penicillin changed the course of history. Penicillin saved thousands of wounded soldiers and civilians during the biggest of the wars, and its discovery laid the foundations of the antibiotic era and subsequent development of other more potent antibiotics.

6.2 VACCINATION

Learning Outcome (e)
Discuss how vaccination can control disease (including the eradication of small pox), limited to vaccination stimulates immunity without causing the disease and vaccination of a high enough proportion of the population can break the disease transmission cycle.

- Vaccination is the intentional administration of an **antigen**, in order to **induce a specific adaptive immune response** that results in production of **memory cells**. It uses the property of **immunological memory**, providing the individual with long-lasting protection against infectious diseases.

- Vaccination provides **active artificial immunity** as the immune response is activated artificially by introducing antigens to the body.

- A vaccine is a preparation of **immunogenic material** used to induce immunity against a pathogen. There are different types of vaccines available.

Type of Vaccine	Advantages	Disadvantages
Live attenuated production of a less virulent / pathogenic bacteria or virus	Closest to a natural infection, thus will elicit strong immune response with low dosage and confer longer-term protection.	Possibility of reversion to virulent form by mutation and cause disease.
Inactivated or killed production of killed pathogenic bacteria or virus with chemicals, heat or radiation	Will not revert to the virulent form and cause disease. Usually do not require refrigeration, and it can be easily stored and transported in a freeze-dried form to make inactivated vaccine accessible.	Elicit weaker immune response, thus may need several doses (i.e. booster shots) to maintain protection.
Subunit production of only antigens on pathogens that can elicit immune response	Will not revert to the virulent form and cause disease since antigens are not allowed to replicate without main part of pathogen.	Takes time to identify antigens found on pathogens that are immunogenic
Toxoid production of chemically modified toxin from pathogenic bacteria such that it is no longer toxic but is still to elicit immune response	Does not contain any pathogen thus there is no risk of reverting to virulent form.	Limited use when bacterial toxin is the main cause of illness but not all pathogenic bacteria produce toxins.
Nucleic Acid production of DNA / RNA coding for antigen of pathogen	Will not revert to the virulent form and cause disease since antigens are not allowed to replicate without main part of pathogen.	Relatively new technology approved for human use. Some RNA vaccines require ultra-cold storage, affecting accessibility.

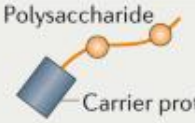
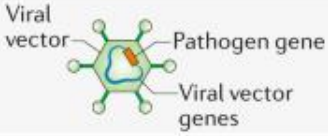
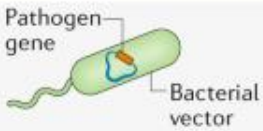
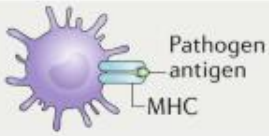
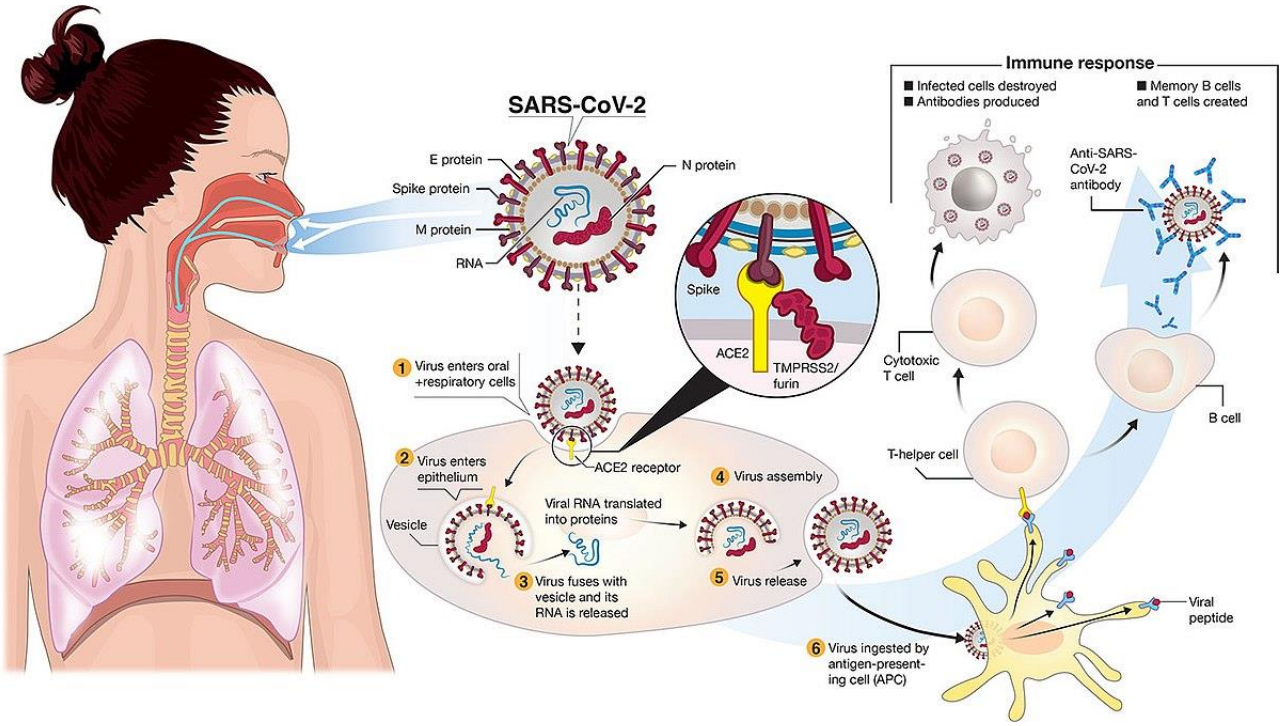
Type of vaccine		Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)		Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism		Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
Toxoid		Diphtheria, tetanus	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)		Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)
Virus-like particle		Human papillomavirus	1986 (hepatitis B)
Outer membrane vesicle		Group B meningococcal	1987 (group B meningococcal)
Protein–polysaccharide conjugate		*Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid	1987 (*H. influenzae* type b)
Viral vectored		Ebola	2019 (Ebola)
Nucleic acid vaccine		SARS-CoV-2	2020 (SARS-CoV-2)
Bacterial vectored		Experimental	–
Antigen-presenting cell		Experimental	–

Fig. 6.2.1: Types of vaccines

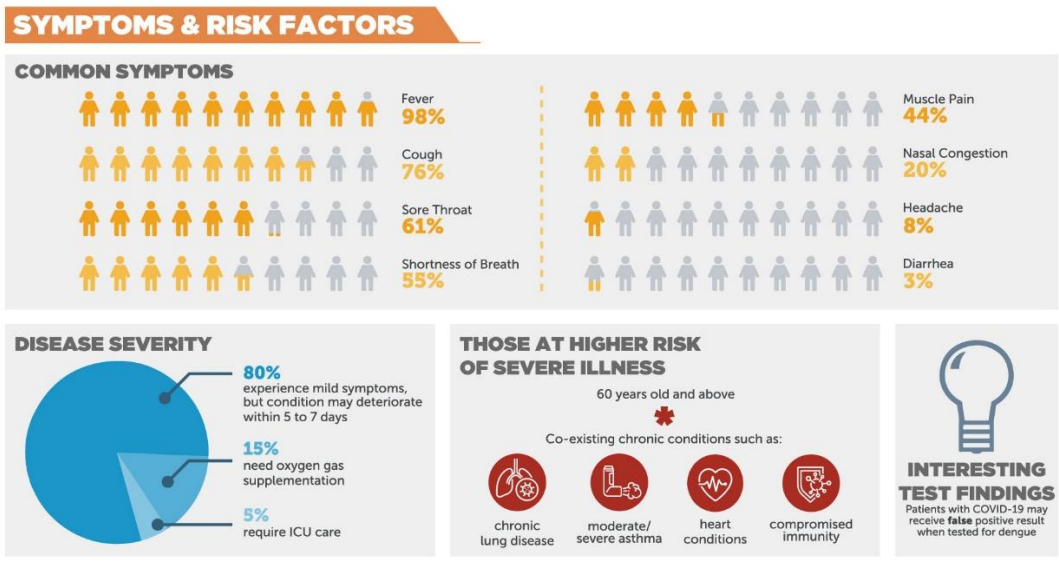
DID YOU KNOW?

COVID-19 Pandemic

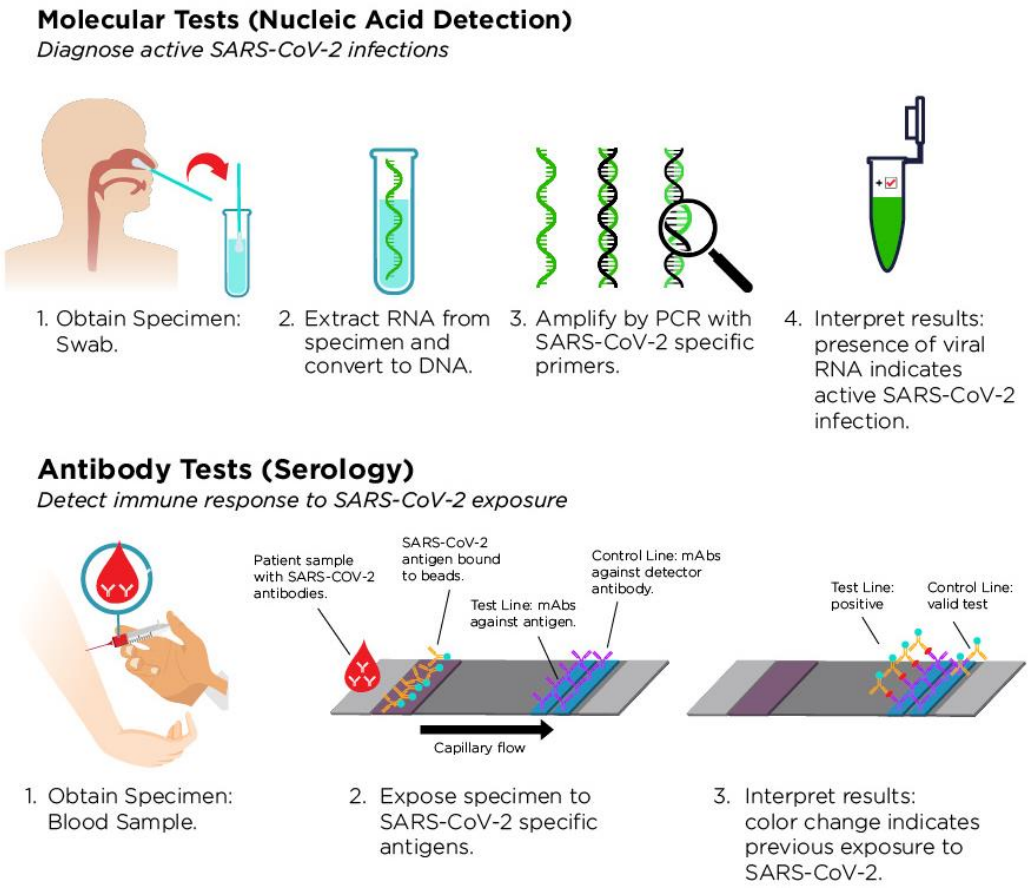


Coronavirus disease 2019 (COVID-19) is a contagious diseased caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The first known case was identified in Wuhan, China, in 2019. The World Health Organization declared a Public Health Emergency of International Concern regarding COVID-19 on 30 January 2020, and later declared a pandemic on 11 March 2020. As of 5 May 2021, more than 154 million cases have been confirmed, with more than 3.23 million deaths attributed to COVID-19, making it one of the deadliest pandemics in history.

Symptoms: Symptoms of COVID-19 are variable, but often include fever, cough, headache, fatigue, breathing difficulties, and loss of smell and taste.



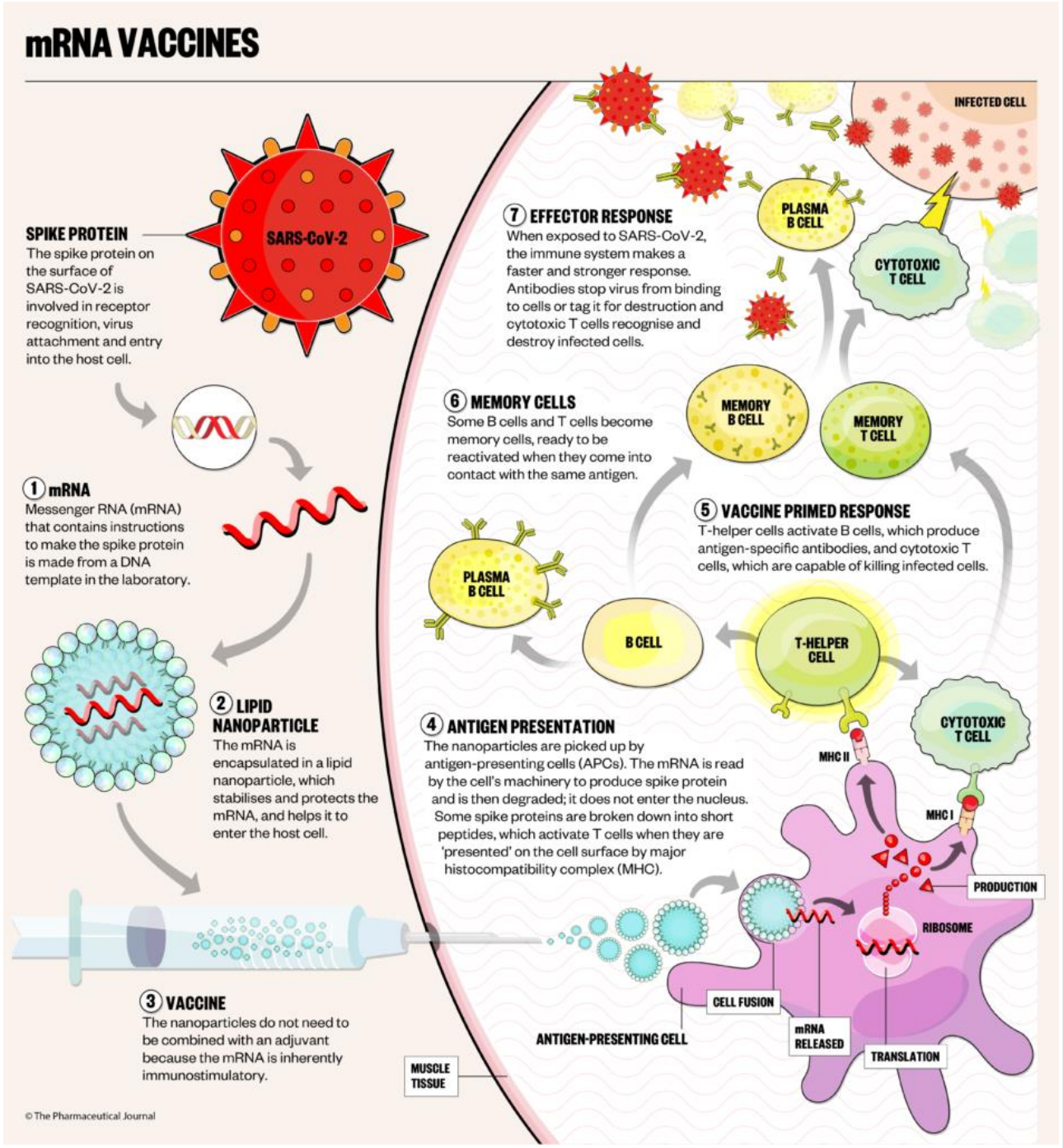
Mode of Transmission: Transmission of COVID-19 occurs through small droplets containing the virus that spread from an infected person as they cough, sneeze or speak.
Detection: Nucleic acid tests via polymerase chain reaction (PCR) and serology tests have been used for testing of COVID-19 infection.



Preventive Measures: Preventive measures include physical or social distancing, quarantining, ventilation of indoor spaces, covering coughs and sneezes, and hand washing. The use of face masks or coverings has been recommended in public settings to minimize the risk of transmissions. Several vaccines have been developed and many countries have initiated mass vaccination campaigns.

Vaccination: After the coronavirus was isolated in late 2019, its genetic sequence was published on 11 January 2020, triggering an urgent international response to prepare for an outbreak and hasten development of a preventive COVID-19 vaccine. Since January 2020, vaccine development has been expedited via unprecedented collaboration in the multinational pharmaceutical industry and between governments. Vaccines must progress through several phases of clinical trials to test for safety, immunogenicity, effectiveness, dose levels and adverse effects of the candidate vaccine.

In December 2020, the mRNA vaccine BNT162b2, developed by Pfizer-NBioTech, was the first COVID-19 vaccine to be approved for use in a country.

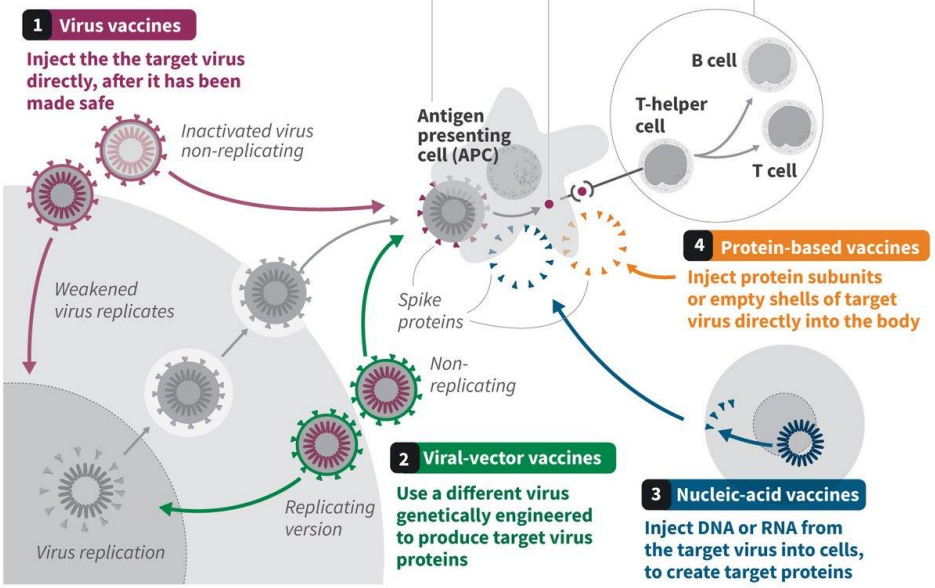

mRNA VACCINES
SPIKE PROTEIN
The spike protein on the surface of SARS-CoV-2 is involved in receptor recognition, virus attachment and entry into the host cell.
SARS-CoV-2
1 mRNA
Messenger RNA (mRNA) that contains instructions to make the spike protein is made from a DNA template in the laboratory.
2 LIPID NANOPARTICLE
The mRNA is encapsulated in a lipid nanoparticle, which stabilises and protects the mRNA, and helps it to enter the host cell.
3 VACCINE
The nanoparticles do not need to be combined with an adjuvant because the mRNA is inherently immunostimulatory.
MUSCLE TISSUE
ANTIGEN-PRESENTING CELL
4 ANTIGEN PRESENTATION
The nanoparticles are picked up by antigen-presenting cells (APCs). The mRNA is read by the cell's machinery to produce spike protein and is then degraded; it does not enter the nucleus. Some spike proteins are broken down into short peptides, which activate T cells when they are 'presented' on the cell surface by major histocompatibility complex (MHC).
CELL FUSION
mRNA RELEASED
RIBOSOME
TRANSLATION
PRODUCTION
MHC I
MHC II
5 VACCINE PRIMED RESPONSE
T-helper cells activate B cells, which produce antigen-specific antibodies, and cytotoxic T cells, which are capable of killing infected cells.
T-HELPER CELL
B CELL
PLASMA B CELL
CYTOTOXIC T CELL
6 MEMORY CELLS
Some B cells and T cells become memory cells, ready to be reactivated when they come into contact with the same antigen.
MEMORY B CELL
MEMORY T CELL
7 EFFECTOR RESPONSE
When exposed to SARS-CoV-2, the immune system makes a faster and stronger response. Antibodies stop virus from binding to cells or tag it for destruction and cytotoxic T cells recognise and destroy infected cells.
PLASMA B CELL
CYTOTOXIC T CELL
INFECTED CELL
© The Pharmaceutical Journal

How vaccines work

The Pfizer Covid-19 vaccine is a **nucleic-acid vaccine**. It uses lab-made SARS-CoV-2 messenger RNA to trigger the body's natural defences

Four main approaches to vaccines

- There are four main approaches towards developing a vaccine against a virus
- All methods rely on APC to initiate the immune response
- APC identifies peptides, snippets of virus protein
- Immune cells will then be enabled to recognise, neutralise virus

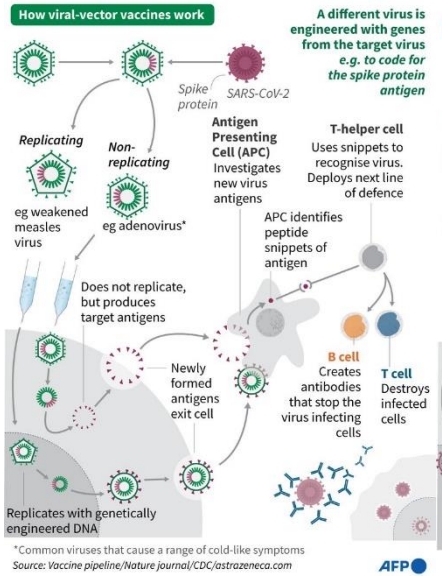


Source: Vaccine pipeline/Nature journal/pfizer.co.uk

AFP

Activating immunity by stealth

The Oxford/AstraZeneca Covid-19 vaccine uses a viral-vector, meaning it delivers the genetic code for a SARS-CoV-2 protein inside the weakened form of a different virus



*Common viruses that cause a range of cold-like symptoms

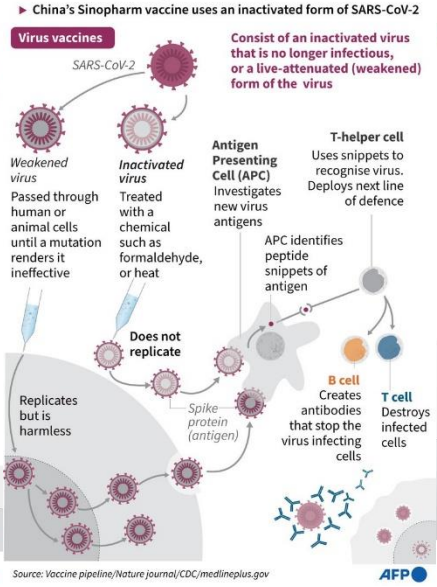
Source: Vaccine pipeline/Nature journal/CDC/astrazeneca.com

AFP

Turning the virus against itself

One of the classic techniques that the majority of licenced vaccinations for human use derive from, such as those for polio and measles.

- China's Sinopharm vaccine uses an inactivated form of SARS-CoV-2

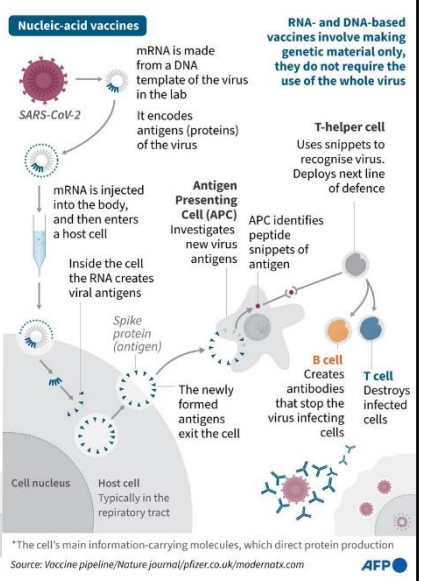


Source: Vaccine pipeline/Nature journal/CDC/medlineplus.gov

AFP

Triggering immunity from genetic code

The Pfizer-BioNTech vaccine is a **nucleic-acid* vaccine.** It uses lab-made SARS-CoV-2 messenger RNA (mRNA) to trigger an immune response



*The cell's main information-carrying molecules, which direct protein production

Source: Vaccine pipeline/Nature journal/pfizer.co.uk/modernatx.com

AFP

6.2.1 Immune Reponse

Upon Vaccination

- A person is vaccinated against a specific disease. The vaccine is no longer able to cause disease, but still possesses the ability to elicit immune response due to presence of characteristic surface **antigen** of the pathogen which be recognized by antigen presenting cells (APC).
- A **primary immune response** occurs and adaptive immune response is elicited. Naïve B and T cells are activated to become effector cells.
- The B cell with B cell receptor that is complementary in shape to the antigen undergoes clonal expansion and differentiation to form either **memory cells** or antibody-secreting plasma cells.

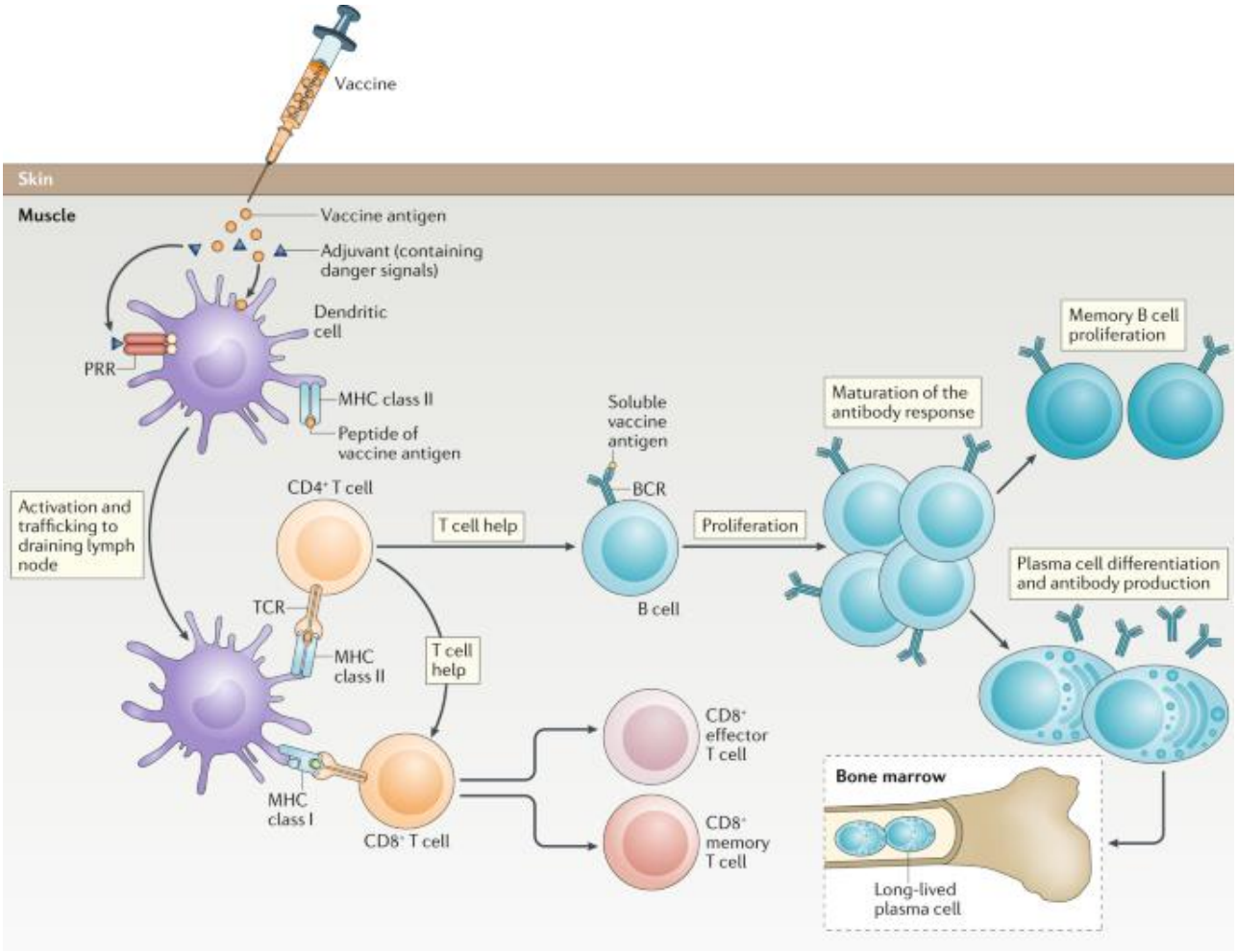


Fig. 6.2.1.1: Immune response upon vaccination

Upon Infection (after Vaccination)

- If the previously vaccinated individual is exposed to the virulent pathogen with the **same** antigen, a **secondary immune response** occurs, which produces a faster and stronger immune response.
- **Memory B cells** will quickly recognize the surface antigen of the pathogen and undergo clonal expansion and develop into **plasma cells**, secreting **high affinity antibodies**.
- Plasma cells produce a large number of antibodies which are able to bind and inactivate the virulent pathogen to prevent them from infecting the healthy host cells in the individual.

6.2.2 Benefits and Risks of Vaccination

Learning Outcome (f)
Discuss the benefits and risks of vaccination.

Benefits of Vaccination

- Vaccines **protect individuals against diseases** by confering immunity to individual even if previously not exposed to a certain pathogen, hence
  - preventing deaths due to diseases.
  - preventing long-term disabilities such as infertility, deafness and blindness due to diseases.
  - extending life expentancy.
- Vaccines **reduce disease severity**. Disease may occur in previously vaccinated individuals. However, in such cases, the disease is usually milder than in the non-vaccinated.
- Some vaccines provide **sterilising immunity**, which **protect the individual against infection**.
- Vaccines can increase **herd immunity**. If a large number individuals in a population are vaccinated and are immune to an infectious agent, fewer susceptible individual will contract the disease. Transmission of the disease within the population is prevented.
  - This allows protection of those in greatest need of protection against infectious diseases, such as pregnant women, cancer patients and the immunocompromised.

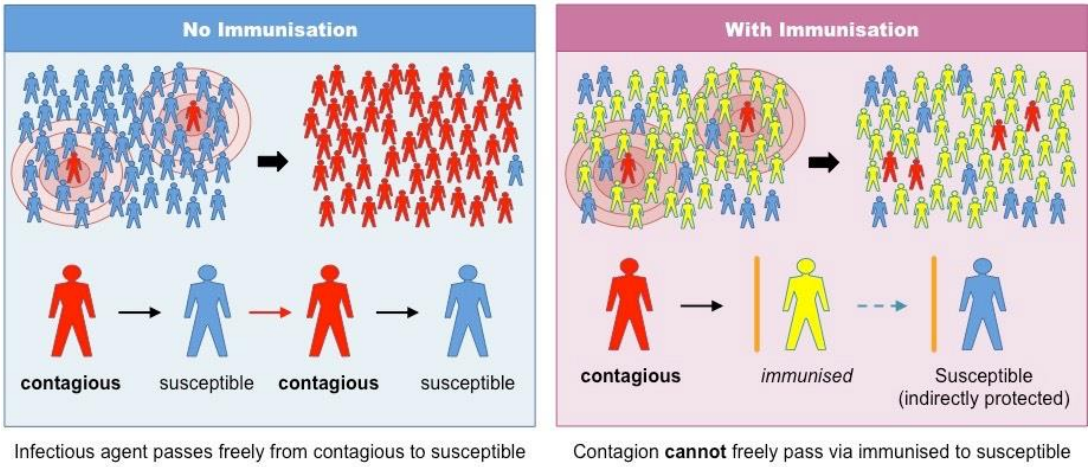


Fig. 6.2.2.1: Herd immunity

- Vaccination enables complete **eradication** of diseases within human population if pathogen relies only on human as host. Eradication requires **high levels of population immunity** in **all regions of the world** over a **prolonged period** with **adequate surveillance** in place.

- **Example: Eradication of Small Pox**
  - Small pox disease is caused by the *variola* virus. The cow pox virus, *vaccinia* virus, belongs to the same family of orthopoxviruses and has the same antigen as the small pox virus but does not cause death (non-virulent) to the infected person.
  - Vaccinating an individual with cow pox virus causes the production of memory B and T cells with receptors complementary to the antigen of the small pox virus. The immune individual

is less likely to die from the disease and will not spread the virus as readily to another individual when infected.

– In 1959, the World Health Organisation (WHO) launched a global vaccination project to eradicate small pox. With strong political motivation and global cooperation for the vaccination project, mass vaccination campaigns were held in many countries, resulting in the eradication of the disease in 1980.

– As a significant proportion of the population was immune through vaccination or infection, this conferred herd immunity. Since the virus depends only on humans as hosts for reproduction, there were insufficient susceptible individuals to allow transmission of the virus, resulting in eradication of the disease.

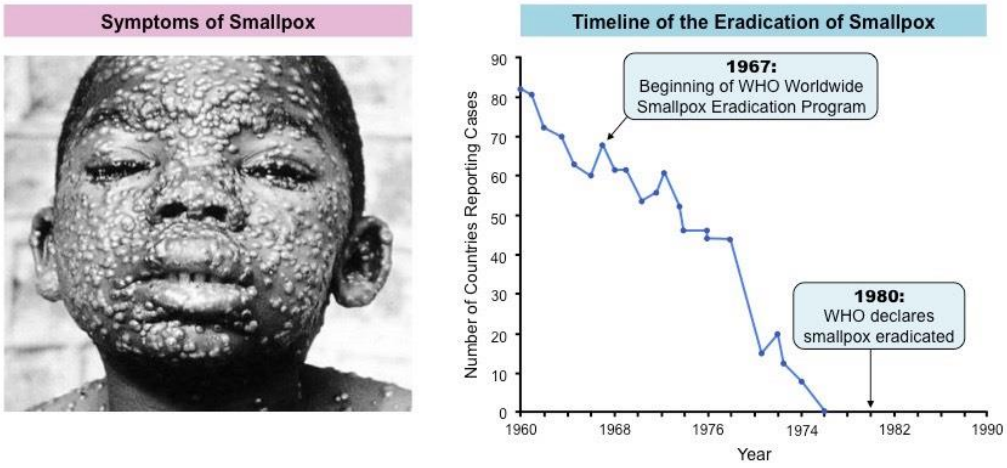


Fig. 6.2.2.2: Eradication of smallpox

- Vaccination reduces healthcare costs due to treatment of diseases.
- Vaccination prevents development of antibiotic resistance by reducing the needs for antibiotics.

Risks of Vaccination

- While live, attenuated vaccines are more effective than inactivated vaccines, they pose the risk of reversion to virulence to cause disease e.g. Chickenpox vaccine.
- Some vaccines may cause side effects that cause a different illness than the disease that the vaccines are designed to prevent. e.g. anaphylaxis (life-threatening allergic reaction), syncope (fainting), rashes.
- Excessive vaccination may reduce the effectiveness of the immune system to respond to new infections.

DID YOU KNOW?

History of Vaccination

One of the first methods for controlling smallpox was **variolation**, a process named after the virus that causes smallpox (variola virus). During variolation, people who had never had smallpox were exposed to material from smallpox sores (pustules) by scratching the material into their arm or inhaling it through the nose. After variolation, people usually developed the symptoms associated with smallpox, such as fever and a rash. However, fewer people died from variolation than if they had acquired smallpox naturally.

Jenner's Experiment

In 1796, intrigued by the fact that milkmaids who had contracted the mild disease cowpox were subsequently immune to the much more severe smallpox, Edward Jenner reasoned that introducing fluid from a cowpox pustule into people (i.e. inoculating them) might protect them from smallpox.

To test this hypothesis, he inoculated an eight-year-old boy with fluid from a cowpox pustule and later intentionally infected the child with smallpox. As predicted, the child did not develop smallpox. He called the procedure **vaccination** (after the Latin term for cowpox, vaccinia), and this term is still used to describe the inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.

1 **(a)** Identify the observation, hypothesis and evidence in Jenner's experiment.

observation .....

hypothesis .....

evidence .....

(b) Discuss the ethical issues concerned with Jenner's experiment

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DID YOU KNOW?

Vaccination in Singapore

The National Childhood Immunisation Schedule (NCIS) comprises childhood vaccinations recommended as the standard of care for protection against vaccine preventable diseases that are of significant healthcare burden to Singapore or would be so without these vaccinations.

National Childhood Immunisation Schedule, Singapore

Vaccination against	Birth	1 Month	3 months	4 months	5 months	6 months	12 months	15 months	18 months	6-7 years^	10-11 years^^
Tuberculosis	BCG										
Hepatitis B	HepB (D1)	HepB (D2)			HepB (D3)#						
Diphtheria, Tetanus, Pertussis			DTaP (D1)	DTaP (D2)	DTaP (D3)				DTaP (B1)		Tdap (B2)
Poliovirus			IPV (D1)	IPV (D2)	IPV (D3)				IPV (B1)		OPV (B2)
Haemophilus influenzae type b			Hib (D1)	Hib (D2)	Hib (D3)				Hib (B1)		
Measles, Mumps, Rubella							MMR (D1)	MMR (D2)##			
Pneumococcal Disease			PCV (D1)		PCV (D2)		PCV (B1)				
Human Papillomavirus		***Recommended for females 9 to 26 years; three doses are required at intervals of 0, 2, 6 months***									

Notes:

BCG	Bacillus Calmette-Guérin vaccine
HepB	Hepatitis B vaccine
Hib	*Haemophilus influenzae* type b vaccine
DTaP	Paediatric diphtheria and tetanus toxoid and acellular pertussis vaccine
Tdap	Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine
MMR	Measles, mumps, and rubella vaccine
OPV	Oral polio vaccine
IPV	Inactivated polio vaccine
PCV	Pneumococcal conjugate vaccine
D1/D2/D3	1st dose, 2nd dose, 3rd dose
B1/B2/B3	1st booster, 2nd booster, 3rd booster
^	Primary 1
^^	Primary 5

#	3rd dose of HepB can be given at the same time as the 3rd dose of DTaP, IPV, and Hib for the convenience of parents.
##	2nd dose of MMR can be given between 15-18 months

ANNEX: GLOSSARY OF TERMS

Terminology	Definition
Active immunity	Immunity resulting from exposure to antigen which provokes the adaptive immunity. There is a delay before the immune response is complete, but it provides ongoing long-term immunity.
Adaptive immune response	The response of antigen-specific lymphocytes to antigen, including the development of immunological memory.
Antibody	A Y-shaped protein with 2 heavy chains and 2 light chains. Each antibody has a unique structure that allows it to bind specifically to a particular antigen. Antibodies are produced by differentiated B cells (plasma cells) in response to infection or immunisation, and bind to and neutralise pathogens or prepare them for uptake and destruction by phagocytes.
Antibody-mediated (humoral) immune response	An adaptive immune response due to antibodies. Adaptive humoral immunity can be transferred to unimmunised recipients by the transfer of serum containing specific antibody (passive immunity).
Antibiotics	Substances that interfere with the metabolism of bacteria, thereby inhibiting the growth of bacteria or killing the bacteria.
Affinity maturation	The increase in affinity of antibodies for their specific antigen as an adaptive immune response progresses, particularly prominent in repeated exposure to the specific antigen.
Antigen	Any molecule that can bind specifically to an antibody or generate peptide fragments that are recognised by a T-cell receptor.
Antigen presentation	The display of antigen on the surface of a cell in the form of peptide fragments bound to MHC molecules recognised by T cells.
Artificial immunity	Immunity that is acquired by a person as a result of medical intervention.
Attenuation	The process by which human or animal pathogens are modified by growth in culture so that they can grow in their host and induce immunity without producing serious clinical disease.
Cell-mediated (cellular) immune response	An adaptive immune response which mainly involves antigen-specific effector T cells.
Chemokine	Small chemoattractant protein that stimulates the migration and activation of cells, especially phagocytic cells and lymphocytes. Chemokines have a central role in inflammatory responses.
Class switching	A recombination process that occurs in activated B cells in which one heavy-chain constant region gene is replaced with another of a different class, resulting in a switch from the production of antibodies of class IgM to the production of IgG, IgA, or IgE classes. Class switching affects the effector functions of the antibodies produced but not their antigen specificity.
Clonal expansion	The proliferation of antigen-specific lymphocytes in response to antigenic stimulation that precedes their differentiation into effector cells. It allows antigen-specific cells to increase in number so that they can effectively combat the pathogen that elicited the response.
Complement system	A system of over twenty blood proteins that sequentially activate each other to amplify the immune response.
Constant region	The part of an immunoglobulin or a T-cell receptor that is relatively constant in amino acid sequence between different molecules. The constant region of an antibody determines its particular effector function.
Cytokine	Any small protein made by a cell that affects the behaviour of other cells.

Cytotoxic T cell	CD8 T cells that have the ability to kill other cells, e.g. virally infected or tumour cells.
Dendritic cell	An antigen-presenting cell that presents antigen to T cells for activation.
Epitope	That portion of an antigen or its fragment that is bound by the antigen-binding site of a given antibody or antigen receptor.
Gene segments	Sets of short DNA sequences at the immunoglobulin and T-cell receptor loci that encode different regions of the variable region of antigen receptors. Gene segments of each type are joined together by somatic recombination to form a complete variable region exon. There are three types of gene segments: V gene segments, D gene segments (in heavy-chain chain loci only), and J gene segments. There are multiple copies of each type of gene segment in the germline DNA, but only one of each type is joined together to form the variable region.
Granuloma	An aggregation of macrophages and immune cells that forms in response to chronic inflammation.
Helper T cell	CD4 T cells that co-operate with other cells in immune responses
Herd immunity	An indirect protection from infectious disease that occurs when a large percentage of a population has become immune to an infection, thereby providing a measure of protection for individuals who are not.
Histamine	An inflammatory mediator secreted by mast cells that promotes vasodilation and increased vascular permeability
Immunity	The ability to resist or eliminate potentially harmful foreign material or abnormal cells.
Immunoglobulin	The protein family to which antibodies and B cell receptors belong.
Inflammation	The local accumulation of fluid, plasma proteins, and white blood cells that is initiated by physical injury, infection, or a local immune response.
Innate immune response	The response to an infection that is due to the presence of, and immediate activation of, the body's innate and relatively non-specific defence mechanisms, such as anatomical barriers, antimicrobial peptides, the complement system, and macrophages and neutrophils carrying nonspecific pathogen-recognition receptors. These mechanisms distinguish between groups of similar pathogens, rather than responding to a particular pathogen. The response does not increase with repeated exposure to a given pathogen.
Leucocytes	White blood cells.
Major histocompatibility complex (MHC)	A protein complex that presents antigenic peptides to T cells.
Natural immunity	Immunity that is acquired by an individual as a natural part of life.
Opportunistic disease	A disease that does not normally affect healthy individuals but can affect individuals with compromised host defences.
Opsonisation	The coating of the surface of a pathogen by antibody and/or complement that makes it more easily ingested by phagocytes.
Pathogens	Microorganisms, such as viruses, bacteria, fungi, protozoa and parasitic worms, which invade the body causing diseases.
Passive immunity	Immunity involving the transfer of antibodies into the body, which binds to their specific antigen if present. This gives instant immunity but only lasts for a few weeks.
Primary immune response	The adaptive immune response that follows the first exposure to a particular antigen.

Secondary immune response	The immune response that occurs in response to a second exposure to an antigen. In comparison with the primary response, it starts sooner after exposure, produces greater levels of antibody, and produces class-switched antibodies. It is generated by the reactivation of memory cells.
Somatic hypermutation	Extensive mutation that occurs in the V region of immunoglobulin genes in activated B cells, resulting in the production of variant immunoglobulins, some of which bind antigen with a higher affinity. These mutations affect only somatic cells and are not inherited through germline transmission.
Somatic recombination or V(D)J recombination	DNA recombination that takes place in developing lymphocytes where different gene segments are recombined into sequences encoding complete protein chains of immunoglobulins and T-cell receptors.
Variable region	The region of an immunoglobulin or T-cell receptor that is the most variable part of the molecule and contain the antigen-binding sites.
Vaccination	The deliberate induction of adaptive immunity to a pathogen by injecting an inactivated or attenuated live form of the pathogen or its antigens (a vaccine).
Vaccine	An immunogenic preparation of inactivated or attenuated live pathogens, or of its antigens, which is injected to provide immunity to that pathogen.