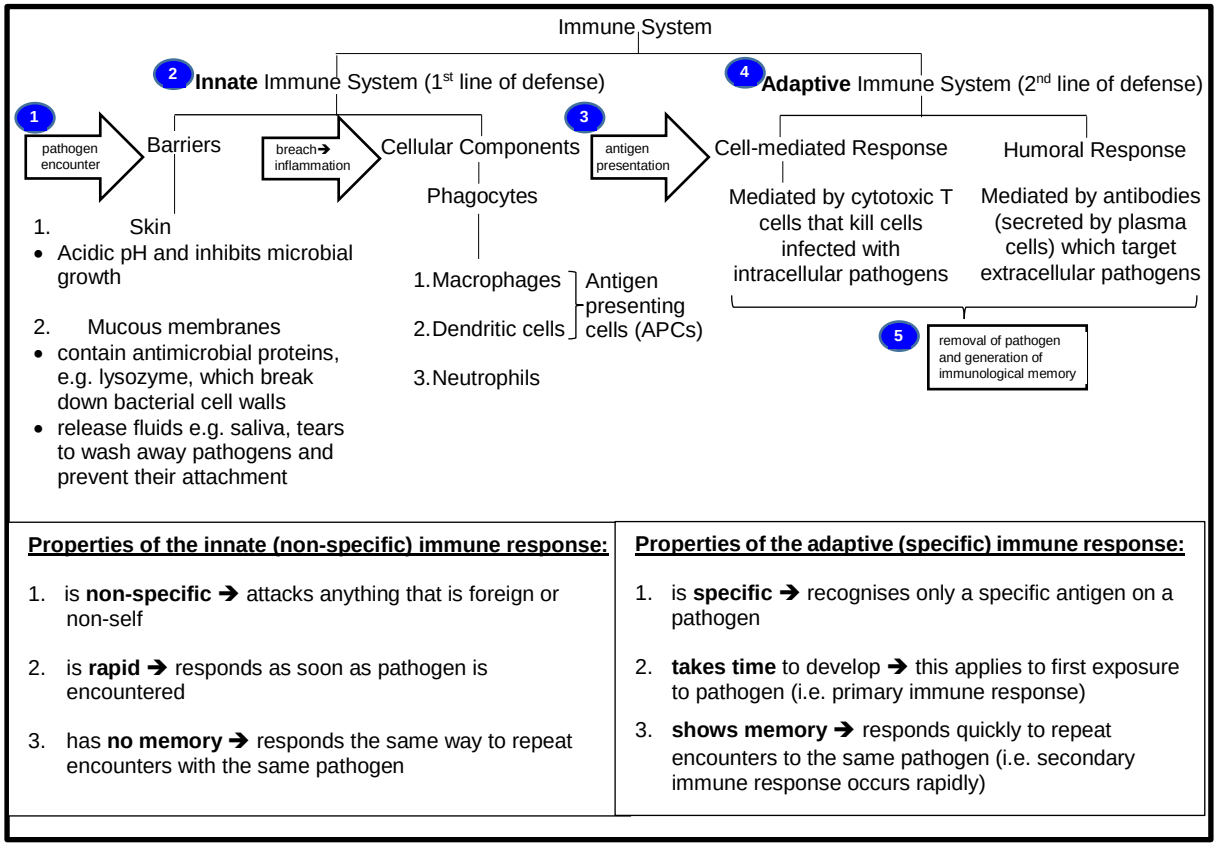
Infectious diseases



Properties of the innate (non-specific) immune response:

1. is **non-specific** ➔ attacks anything that is foreign or non-self
2. is **rapid** ➔ responds as soon as pathogen is encountered
3. has **no memory** ➔ responds the same way to repeat encounters with the same pathogen

Properties of the adaptive (specific) immune response:

1. is **specific** ➔ recognises only a specific antigen on a pathogen
2. **takes time** to develop ➔ this applies to first exposure to pathogen (i.e. primary immune response)
3. **shows memory** ➔ responds quickly to repeat encounters to the same pathogen (i.e. secondary immune response occurs rapidly)

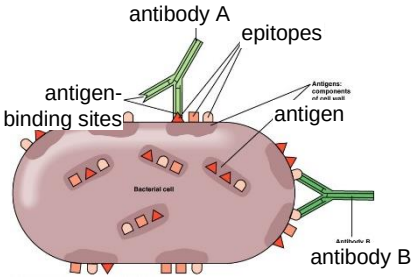
* **Infection:** process where a pathogen **invades and multiplies** in a host and causes **ill health** in the host

* **Pathogens:**

1. microorganisms that **cause disease** (impair normal function) by **invading and multiplying** in the host
2. e.g. bacteria, viruses, fungi, worms and protozoa
3. can be intracellular (i.e. in cells) or extracellular (i.e. in blood, tissue fluid and lymph i.e. in the humour)
4. have antigens on their surface

* **Antigens:**

1. many different types can be found on one pathogen
2. triggers an immune response when specific parts of the antigen called epitopes are recognised by immune cells or antibodies



* **Epitopes:**

1. parts of a single antigen
2. each have a specific conformation complementary in shape and charge to a specific antigen-binding site of an antibody or T cell receptor or B cell receptor

* **Cells of the Immune System:**

1. **Phagocytes**

a) **macrophages**
➔ an antigen presenting cell (APC) which resides in tissues
➔ are produced when monocytes in the blood enter tissues and differentiate

b) **dendritic cells**
➔ an antigen presenting cell (APC) which resides in tissues

c) **neutrophils**
➔ found in blood

2. **Lymphocytes**

T lymphocytes/cells	B lymphocytes/cells
Originate from haematopoietic stem cells in bone marrow but **differentiate in the thymus** to form naïve T cells	Originate from haematopoietic stem cells in the bone marrow and **differentiate in the bone marrow** to form naïve B cells
Each T cell has a specific T cell receptor (TCR) on its surface	Each B cell has a specific B cell receptor (BCR) on its surface
A TCR can only recognise and bind to a **specific, complementary <u>processed</u>** peptide of a **peptide-MHC complex** on an antigen-presenting cell (APC)	A BCR can recognise and bind to a **specific complementary <u>unprocessed</u> antigen of a pathogen.** Antigen binding site of the BCR and the specific antibody produced in response to the antigen are the same.
naïve T cell → when activated by APC → effector T cells / memory T cells; effector T cells → helper T cells ($CD4^+$ T cells) / cytotoxic T cells ($CD8^+$ T cells)	naïve B cell → when activated by helper T cell → plasma B cells / memory B cells; plasma B cells → produce → antibodies
When a specific naïve T cell is activated by a specific antigen presenting cell, it undergoes **clonal expansion and differentiation** to form effector T cells (i.e. helper T and cytotoxic T cells) and memory T cells	When a specific naïve B cell is activated by a specific helper T cell, it undergoes **clonal expansion and differentiation** to form effector B cells (i.e. plasma B cells) and memory B cells
Helper T cell: activates naïve B cell so that it can undergo clonal expansion and differentiation	**Plasma B cell**: produces **antibodies** which are involved in the **humoral response** and protect against **extracellular** pathogens and toxins secreted by pathogens. Plasma B cells have an extensive network of rough endoplasmic reticulum needed for synthesis of large amounts of antibodies (globular proteins) which are secreted by exocytosis.
Cytotoxic T cell: involved in **cell-mediated response** and hence protects against **intracellular** pathogens by killing cells that contain pathogens	
Memory T cell: When re-exposed to the **same** pathogen, memory T cells will recognise it and undergo faster clonal expansion & differentiation into effector T cells, mounting a **faster and stronger secondary immune response** (compared to the primary immune response)**.** Memory cells also confers **long term immunity** to a specific pathogen.	**Memory B cell**: When re-exposed to the **same** pathogen, memory B cells will recognise it and undergo faster clonal expansion & differentiation into antibody-secreting plasma B cells, mounting a **faster and stronger secondary immune response** (compared to the primary immune response)**.** Memory cells also confers **long term immunity** to a specific pathogen.

* **<u>5 steps in immune response</u>**:

1 Pathogen encounter → 2 Innate immune response → 3 Antigen presentation → 4 Adaptive immune response → 5 Removal of pathogen and immunological memory

1 * **Physical and chemical barriers** of the innate immune system **prevent entry** of pathogens
When these barriers are breached → the pathogen enters the body tissues

2 This will cause phagocytes such as **macrophages** in the body tissues
(A) to **engulf the pathogens** by **phagocytosis** and
(B) to **induce inflammation** → aim: to recruit more phagocytes

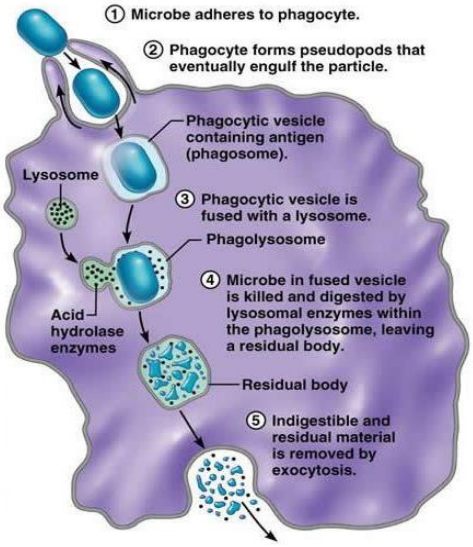


Fig: Phagocytosis

* (A) **<u>Phagocytosis by macrophage:</u>**

1. Bacterium becomes attached to membrane evaginations called **pseudopodia**
2. Bacterium is ingested, forming **phagosome** which then fuses with **lysosome** forming **phagolysosome**
3. Lysosomal enzymes digest the captured material and digested products are released from the cell by **exocytosis**

* (B) **<u>How are more phagocytes recruited during inflammation?</u>**
Pathogen triggers **macrophages** at site of infection (i.e. infected tissues) to **release** signalling molecules, **chemokines** and **cytokines** which (see table)

Effect of chemokines and cytokines on blood vessels	Effects
Vasodilation: widening of blood vessels → increases blood flow (causing redness, heat)	More phagocytes can be carried to site of infection from the bloodstream
Vasopermeability: increase permeability of blood capillaries	More phagocytes can be recruited to site of infection from bloodstream
	Fluid accumulation at site of infection (causing swelling)
	Release of inflammatory mediators (causing pain)

Phagocytes that are recruited to site of infection during inflammation are
(1) **macrophages** → carry out phagocytosis and clear pathogen or function as an antigen presenting cell (APC)
(2) **neutrophils** →found in blood→migrate to site of infection→carry out phagocytosis and then die and form pus

* **<u>If a pathogen evades the innate immune system</u>** (i.e. the barriers and cellular components of the innate immune system are breached)
→ the **adaptive immune system** is **activated** by **antigen presentation**.

3 * **Antigen Presentation:**

1. **Antigen uptake**:**Phagocytic APC engulfs** pathogen
2. **Processing of antigen**:antigen is 'cut up' into short peptides inside phagolysosme
3. Peptides of antigen bind to MHC protein forming **peptide-MHC complex**
4. Peptide-MHC complex is transported via vesicles fuses with the cell surface membrane of the APC.
5. The peptide on the MHC complex is now ready for **presentation** to the **complementary T cell receptor** (TCR) on a **specific naïve T cell**. (See (*****) next to **pt. 2**. below to see what occurs next)

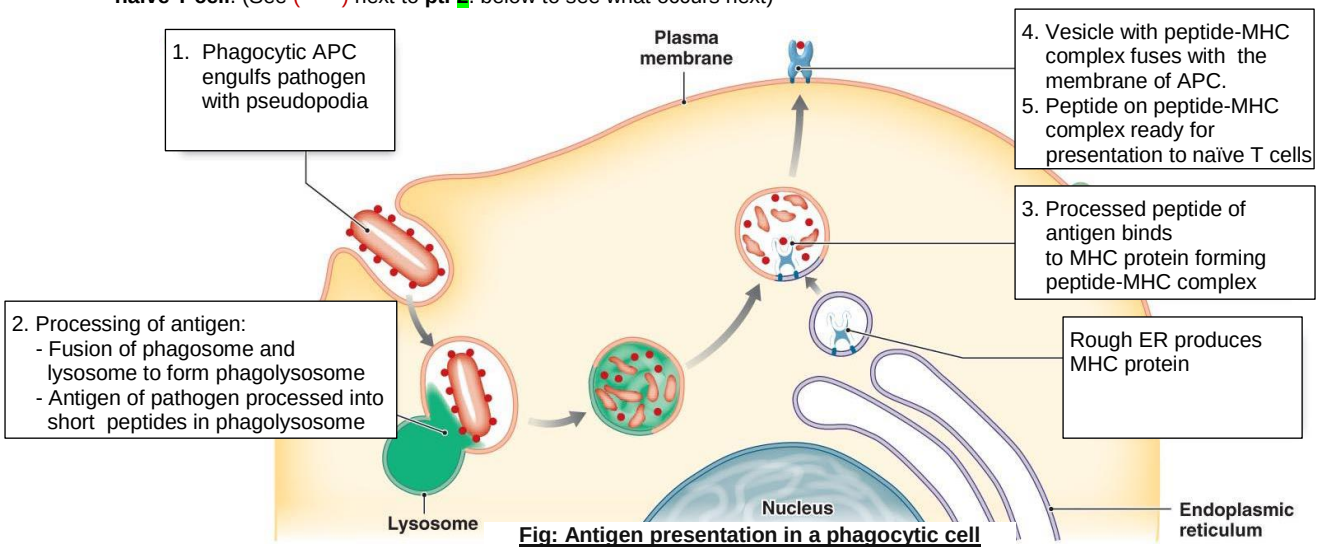


Fig: Antigen presentation in a phagocytic cell

3 *** How naïve cells become activated and the adaptive immune response:**

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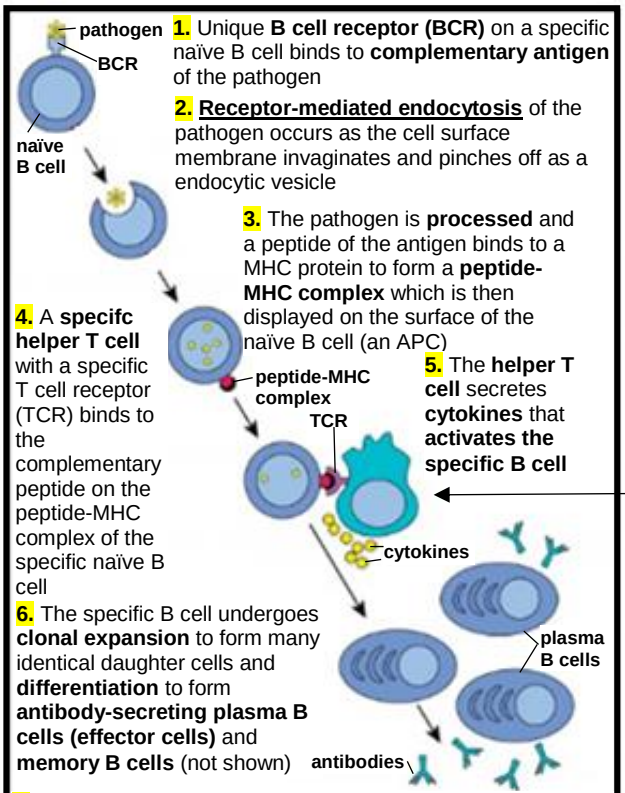


1. Unique **B cell receptor (BCR)** on a specific naïve B cell binds to **complementary antigen** of the pathogen

2. **Receptor-mediated endocytosis** of the pathogen occurs as the cell surface membrane invaginates and pinches off as a endocytic vesicle

3. The pathogen is **processed** and a peptide of the antigen binds to a MHC protein to form a **peptide-MHC complex** which is then displayed on the surface of the naïve B cell (an APC)

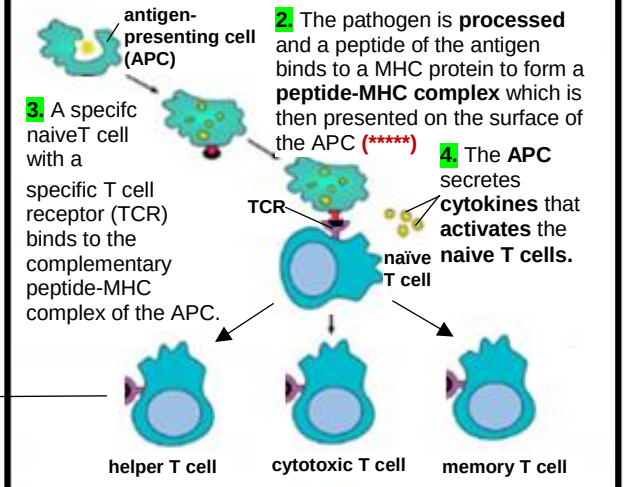
4. A **specifc helper T cell** with a specific T cell receptor (TCR) binds to the complementary peptide on the peptide-MHC complex of the specific naïve B cell

5. The **helper T cell** secretes **cytokines** that **activates the specific B cell**

5 **6.** The specific B cell undergoes **clonal expansion** to form many identical daughter cells and **differentiation** to form **antibody-secreting plasma B cells (effector cells)** and **memory B cells** (not shown)

7. The antibodies then destroy and clear the **extracellular** pathogen by **neutralisation, opsonisation and agglutination** (pg.4)

8. If the body is re-exposed to the **same** antigen, the memory B cells, rapidly undergo **clonal expansion and differentiation into many plasma B cells** that can rapidly manufacture large quantities of antigen **specific antibodies**



1. An antigen presenting cell (e.g. dendritic cell) forms pseudopodia around the pathogen which fuse and encloses the pathogen in a phagosome by **phagocytosis**.

2. The pathogen is **processed** and a peptide of the antigen binds to a MHC protein to form a **peptide-MHC complex** which is then presented on the surface of the APC **(*****)**

3. A specifc naiveT cell with a specific T cell receptor (TCR) binds to the complementary peptide-MHC complex of the APC.

4. The **APC** secretes **cytokines** that **activates** the **naive T cells.**

5. The specific activated naive T cells undergo **clonal expansion** to form many identical daughter cells and **differentiation** into **effector T cells** (**cytotoxic T cells** and **T helper cells**) and **memory T cells**

6. The **helper T cells** secrete cytokines which (a) **activate naïve B cells** which form **plasma B cells** and (b) **stimulate macrophages** to attack infected cells.(not shown)

7. The **cytotoxic T cells kill cells infected with intracellular pathogens like viruses** by producing **perforins** and **granzymes** (see pg. 4)

8. If the body is re-exposed to the **same** antigen, the memory T cells rapidly undergo **clonal expansion and differentiation into many helper T cells** and **cytotoxic T cells**.

*** Clonal selection** is a process proposed to explain how a single B or T cell that recognizes an antigen that enters the body is **selected** from the pre-existing cell pool of differing antigen specificities and then reproduced to generate a **clonal** cell population that eliminates the antigen.

*** Clonal expansion and differentiation:** refers to the repeated division of cells by **mitosis** and specialisation of cells due to **differential switching on of genes** respectively.

* **Humoral response:**

Protect against **extracellular** pathogens and toxins (i.e. those found in the blood)

1. **Neutralisation** of pathogen:

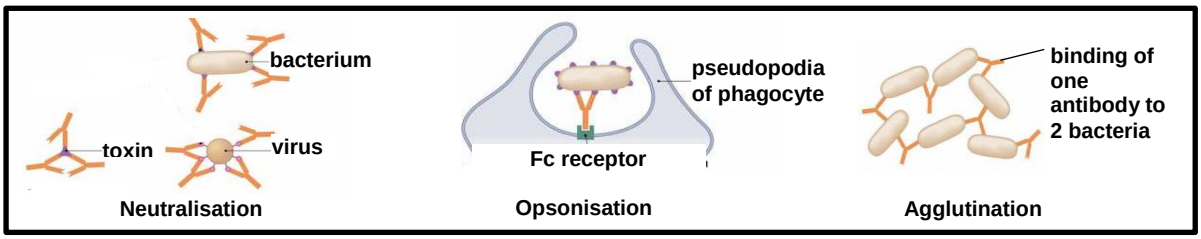
binding of antigen binding site of antibody to pathogen/toxin → prevents binding of pathogen/toxin to host cell receptor and hence prevent entry into host cell

2. **Opsonisation** to facilitate phagocytosis:

after binding of antigen binding site of antibody to pathogen → binding of Fc portion of antibody to Fc receptors on phagocyte → signals from the Fc receptors on the phagocyte promotes phagocytosis of the pathogen

3. **Agglutination**

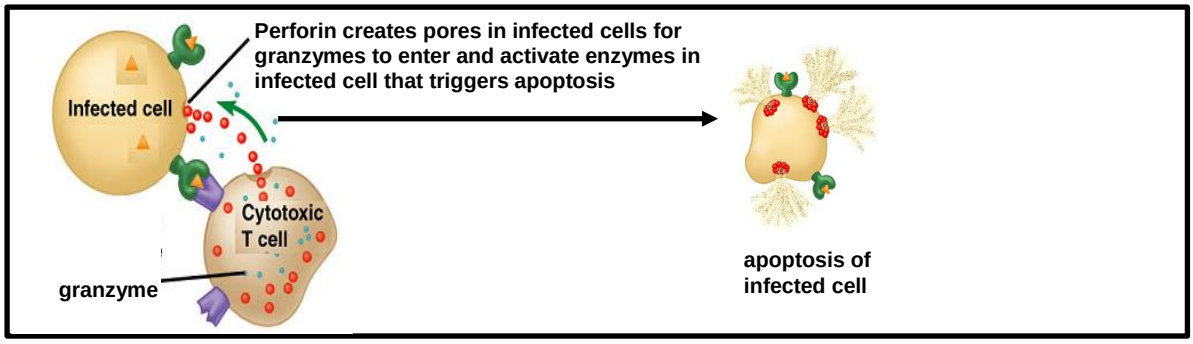
binding of each antigen binding site of an antibody to 2 pathogens simultaneously → clumping or aggregation of pathogens → promotes phagocytosis of the pathogen (Thus agglutination reduces the number of infectious units to be dealt with and prevents the pathogen from spreading)



* **Cell-mediated response:**

Protect against **intracellular** pathogens by killing cells that contain pathogens (e.g. a virally infected cell)

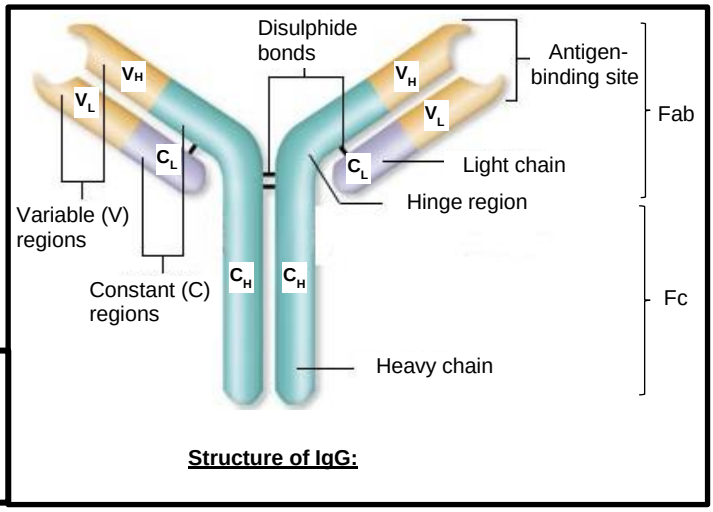
TCR on cytotoxic T cells recognise infected target cells which display short peptides from antigen of pathogen presented on a MHC → Cytotoxic T cells release
1. **perforins** that make pores in the infected cell's membrane and
2. **granzymes** that diffuse through the pores and activate enzymes in the cell that triggers apoptosis of the virus infected cell



* **Antibodies:**

1. aka **immunoglobulins**
2. are **globular** proteins **secreted** by **plasma B cells**
3. made up of 4 polypeptide chains
   ➔2 identical light chains and 2 identical heavy chains
   ➔ hence has a quaternary structure which is held together by ionic and hydrogen bonds, hydrophobic interactions (not shown in figure) and disulfide bridges between the R groups of the amino acids of the 2 heavy chains and the heavy chains and the light chains

Fab: Fragment antigen-binding
Fc: Fragment crystallisable
V_H: variable region of heavy chain
V_L: variable region of light chain
C_H: constant region of heavy chain
C_L: constant region of light chain



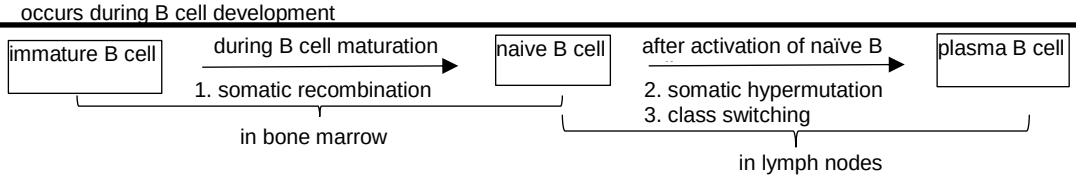
Structure of IgG:

* **Antibody Structure and Function:**

Structure	Function
Antigen binding site of a specific antibody is **complementary in shape** to a **specific epitope of an antigen** due to the precise folding of the **variable heavy and light chains** that gives rise to its unique **3D structure**	Hence antibodies can carry out **neutralisation** by **binding to specific epitope of an antigen of pathogen thus preventing pathogen from binding to host cell receptors** and infecting the host cells and at the same time facilitates **agglutination**
Fc region of antibody/constant region of heavy chain has a conformation that is complementary in shape to **Fc receptors on phagocytes**	Hence **opsonisation** can occur as once antibodies bind to the pathogen, **Fc regions of antibodies bind to Fc receptors of phagocytes** and **promote phagocytosis**
Disulfide bridges between **heavy and light chains** / **two heavy chains**	This gives **stability** to the **quaternary structure** by holding the heavy and light chains together / heavy chains together
Each antibody has a **hinge region**	This give antibody **flexibility when binding** to epitopes/antigen/pathogen that are **variable distances apart**
Each has **two antigen binding sites**	Each antibody can bind to **two epitopes/antigens** at the **same time** which will cause pathogens to **aggregate/agglutinate/clump tog**ether to facilitate clearance by macrophages;
Constant region of **heavy chains**	determine the **class of antibody** thus their **different functions**

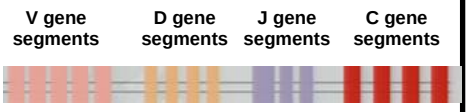
*** Antibody diversity:**

1. involves 3 genes: 2 genes coding for light chains (kappa and lambda) &1 gene coding for heavy chains
2. due to a. somatic recombination b. somatic hypermutation c. class switching
3. occurs during B cell development



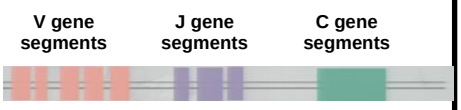
Heavy chain gene

1. has many different variable (V), diversity (D), joining (J) and constant (C) gene segments
2. the V, D and J gene segments contribute toward the variable region of the heavy chains (V_H)
3. The C segments contribute to the constant region of the heavy chain (C_H)



Light chain gene

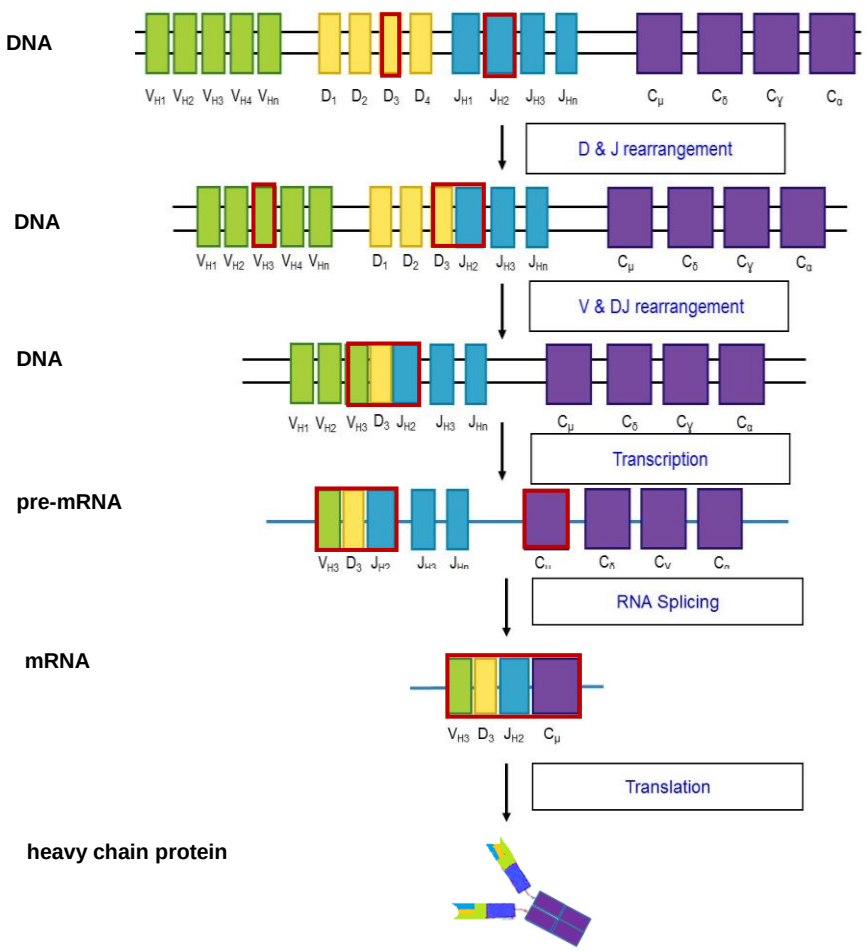
1. has many different variable (V), joining (J) and constant (C) gene segments
2. the V and J gene segments contribute toward the variable region of the heavy chains (V_L)
3. The C segments contribute to the constant region of the light chain (C_L)



* **Somatic recombination:**

1. There are **multiple gene segments** at heavy and light chain genes
2. **Somatic recombination** is a form of **DNA rearrangement** where various gene segments are joined together **randomly**, and some **intervening segments** are **enzymatically removed** followed by rejoining of remaining sequences.
3. At the Ig **heavy** chain gene locus**, one V segment, one D segment** and **one J segment** are **randomly** joined to form a single VDJ exon
4. At the Ig **light** chain gene locus, **one V segment** and **one J segment** are **randomly** joined to form a single VJ exon

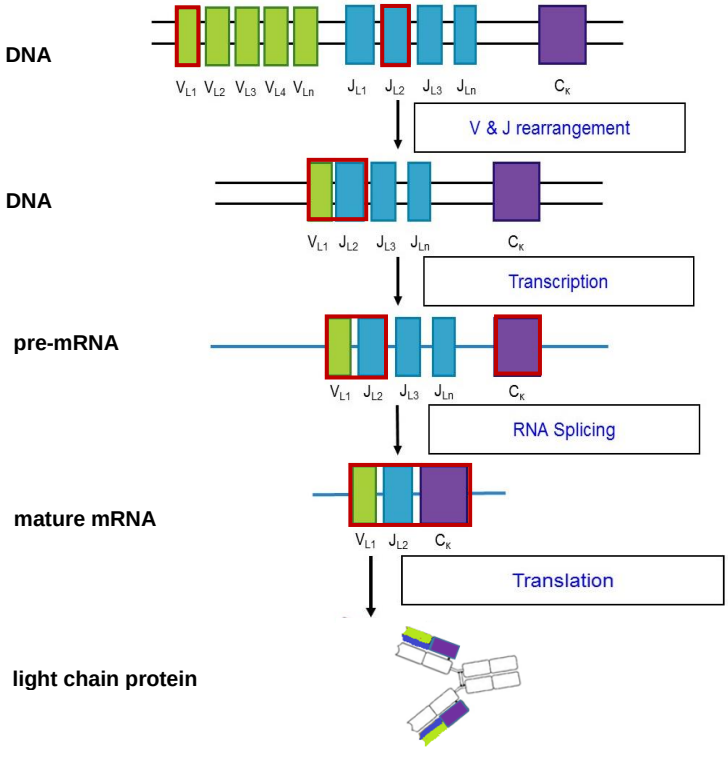
1. Somatic recombination in heavy chain gene



VDJ recombination occurs
➔at the **heavy chain gene**
➔during B cell maturation in the bone marrow

1. During **D and J rearrangement, one D segment** and **one J segment** are joined and the intervening sequences are enzymatically removed
2. During **V and DJ rearrangement, one V segment** is joined to the **DJ segment** and the intervening sequences are enzymatically removed
3. The DNA segment then undergoes **transcription**
4. The resulting pre-mRNA undergoes **RNA splicing** during which the introns are excised and the VDJ exon and C exon are joined
5. The mature mRNA with the VDJC exons joined enters the cytoplasm where it undergoes **translation** to form the **specific heavy chain protein** with the specific V, D, J and C domains

2. Somatic recombination in light chain gene



VJ recombination occurs
➔at the **light chain gene**
➔during B cell maturation in the bone marrow

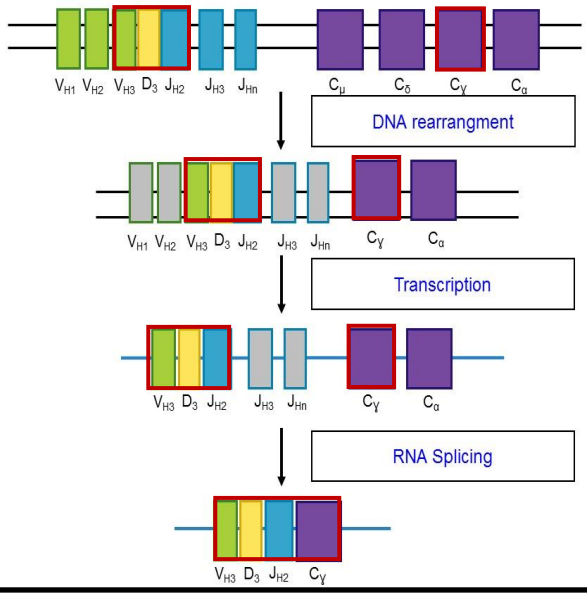
1. During **V and J rearrangement,** one V segment and one J segment are joined and the intervening sequences are enzymatically removed
2. The segment then undergoes **transcription**
3. The resulting pre-mRNA undergoes **RNA splicing** during which the introns are excised and the VJ exon and C exon are joined
4. The mature mRNA with the VJC exons joined enters the cytoplasm where it undergoes **translation** to form the **specific light chain protein** with the specific V, J and C domains

* **Somatic hypermutation:**

1. **Somatic hyper-mutation** is **random point mutation** in the rearranged **VDJ / VJ regions** in activated B cells
2. Further diversifies **variable regions** of antibody for antigen binding (due to slight amino acid differences which result due to the mutation)
3. It occurs during clonal expansion of the activated B cells
3. Some point mutations result in the B cells expressing **low affinity Ig chains** on their cell surface membrane and some point mutations result in the B cells expressing **higher affinity Ig chains** on their cell surface membrane
4. B cells that express higher affinity BCR on their cell surface membrane are **selected for** clonal expansion & differentiation. This is called **affinity mutation**
5. The resulting plasma B cells and memory B cells will have BCRs with higher affinity antigen binding sites for a specific antigen. The plasma B cells will also produce antibodies with higher affinity antigen binding sites for a specific antigen.

* **Class Switching:**

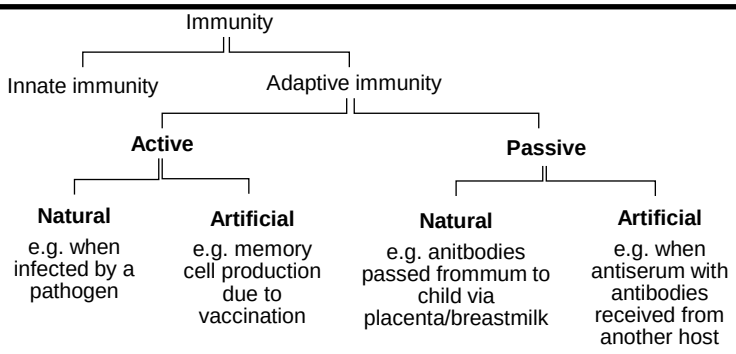
1. **Class switching** is **DNA rearrangement** at the **constant gene segment** of the **heavy chain gene locus** in activated B cells
2. Allows for the production of antibodies with **same antigen binding site**, but **different function**



Heavy chain gene
➔ VDJ recombination complete
➔ occurs after B cell activation

1. Before class switching, V_H DNA linked to Cμ. Hence Ig M antibodies are produced.
2. During class switching, **DNA rearrangement** occurs and V_H DNA becomes linked to another constant gene segment of the heavy chain gene locus, for example, Cγ Hence Ig G antibodies are produced
3. Only the constant region of the heavy chain (C_H) of the antibody changes, due to the change of C gene segment from Cμ to Cγ. Hence Ig G is produced instead of Ig M.

* **Active and Passive Immunity:**

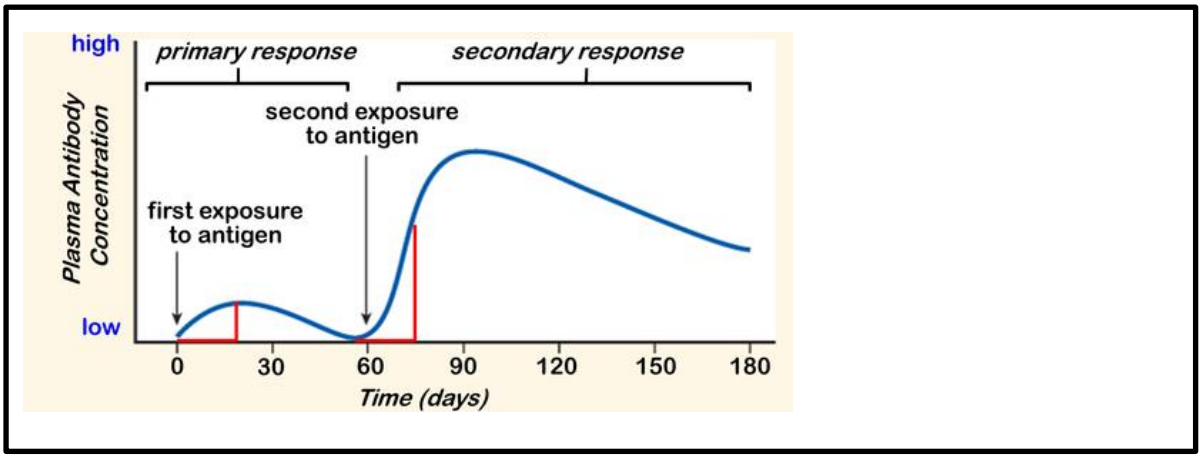


	Active immunity	**Passive immunity**
Immune response	Yes	No
Antibody production	Produced by individual's own immune system in response to antigens	Transferred to recipient without the participation of the recipient's immune system
Duration	Long term immunity conferred since memory cells are formed after primary immune response	Short-lived since there is no formation of memory cells

* **Immunological Memory:**

- When naïve B cells are activated, clonal expansion and differentiation will produce plasma B cells and memory B cells. M**emory B cells**, can **remain in the body for years** (or even a lifetime).
- Immunological memory is established to ensure rapid re-induction of antigen-specific antibodies on subsequent encounters with the SAME pathogen, thus providing long-lasting protection against it.
- Since the individual does not presents exhibit any symptoms of infection upon exposure, the individual is said to be **immune**.

Primary immune response	**Secondary immune response**
Presence of **lag period**: 3-6 days between encountering antigen & production of antibody (time required for clonal expansion & differentiation of T cells to effector T cells, then B cells to plasma B cells)	Response is **faster**: within hours (memory B & T cells can be reactivated easily, leading to a faster response to re-exposure to the same pathogen)
Response is **weaker**: antibody concentration rises gradually, and peaking at a lower level after a longer time	Response is **stronger**: antibody concentration peaks at much higher level and quicker
No Memory	Has memory: Confers long term immunity to the SAME pathogen if it is encountered again



* **Vaccination:**

- **Vaccination**: the intentional **administration of an antigen**, usually a harmless form of a pathogen in order **to induce a specific adaptive immune response** that protects the individual against later exposure to the pathogen due to the production of memory cells. The individual should not develop disease symptoms.

- A form of **artificial active immunity**: immune response is activated artificially by introducing antigens into the body to initiate primary immune response

- Uses the property of **immunological memory** to provide long-lasting protection against infectious diseases so that when exposed to the actual pathogen, the memory cells trigger a **secondary immune response** that is **faster and stronger**

- **How vaccines cause primary immune response (general)**

➔ The vaccine contains an **attenuated/ weakened/ killed form** of pathogen:
➔ The modified pathogen is **no longer able to cause disease** (removal of virulence gene which allows pathogen to successfully replicate & cause disease in host) but it still retains **immunogenic effect** (ability to **elicit an immune response**) because characteristic **surface antigens** of the pathogen are retained and can be **recognised by APCs**
➔ Adaptive immune response occurs: specific naïve B and T cells are activated to become effector and memory B and T cells respectively (i.e. **primary immune response** is triggered)

- **How vaccines protect from actual pathogen**

➔ If the previously vaccinated individual is exposed to the virulent pathogen with the SAME antigen, memory B & T cells will quickly recognize the surface antigen of the pathogen and mount a **faster and stronger secondary immune response**
➔ Memory B cells **rapidly undergo clonal expansion and differentiation** and develop into antibody-secreting plasma B cells
➔ The plasma B cells produce a large number of antibodies which are able to bind to & inactive the virulent pathogen to prevent it from infecting healthy host cells in the individual
➔ Hence vaccines confer **long term immunity**. However because memory cells may not survive a life time, **booster shots** may be required.

* **Example of a type Vaccine:**

	Live, attenuated vaccine
Definition	**Attenuation**: weakening of pathogenic bacteria/ virus by making it less virulent, done through the removal of the virulence gene
Advantages	Closest thing to a natural infection ➔ will elicit a **strong** immune response with just a **small dosage** & confer **longer-term protection**
Disadvantages	• Possibility of **reversion to virulent form** by mutation ➔ can then cause disease • Needs to be **refrigerated** to stay potent

*** Benefits and Risks of Vaccinations:**

Benefits	Risks
• Vaccines protect **individuals** against disease • Deaths due to illness can be prevented • Long-term disabilities (e.g. infertility, deafness, blindness) due to diseases can be prevented • **Herd immunity** - If there are **sufficient individuals in a population (95%)** who are **immunised** due to vaccinations, **transmission of the disease** in a community is **less likely** due to the **low chance** of a an individual who is **not vaccinated** (e.g babies, elderly) **coming into contact with an infected individual.** Hence the **unvaccinated are protected** because **transmission is prevented.** • Some diseases may be **completely eradicated** by vaccination, reducing human suffering & future costs of treatment e.g. Smallpox (unique circumstances) - Human was the only host - Virus did not mutate - Infected people easily identified & isolated, no symptomless carriers - Compulsory live, attenuated vaccine that was also heat stable; no boosters required (note: some people are unsuitable for vaccines)	• While live, attenuated vaccines are more effective than inactivated vaccines, they pose the risk of **reversion to virulence** to cause disease • Some people may be **allergic** to components in vaccines • Immunity developed after vaccination **may not be as effective as natural immunity** due to a real infection. (Hence booster shots may be needed.) • Some pathogens **mutate very quickly** and a new vaccine is need every year • Excessive vaccination may **reduce effectiveness of immune system** to respond to new infections

*** Treatment of Bacterial Infections with Antibiotics:**

- **Definition**
  - Natural substances obtained from microorganisms e.g. certain fungi & bacteria
  - Inhibit growth of bacteria or kill bacteria by disrupting metabolism of prokaryotic cells (make use of differences between prokaryotic & eukaryotic cells to target bacteria without harming human cells)

- **Role**
  - Prevent spread of bacteria within the body and hence aid recovery
  - Prevent death as consequences may be fatal without treatment
  - Prevent transmission of disease from individual to individual in a population

- **Types**
  - Bacteriostatic: inhibits cell division of bacteria
  - Bactericidal: kills bacteria when bacteria are in the process of undergoing cell division by binary fission
  - Can disrupt different metabolic pathways e.g. cell wall synthesis, protein synthesis (translation), nucleic acid synthesis

- **Disrupting cell wall synthesis**
  - Targets bacterial cell wall (usually made of peptidoglycan) because humans do not have cell walls
  - E.g. penicillin (a ß-lactam antibiotic: four-membered, nitrogen containing ß-lactam ring structure)
    1. Bactericidal: only effective when bacteria is growing/ making new cell wall as it disrupts cell wall synthesis
    2. Penicillin acts as a competitive inhibitor & binds to active site of transpeptidase
    3. Inhibition to formation of cross-links between adjacent chains of peptidoglycan
    4. Bacterial cell wall becomes weakened when bacterial cells carry out division
    5. An increase in turgor pressure against the weakened cell wall causes bacteria to swell & lyse

- **Disrupting protein synthesis**
  - Ribosomes of prokaryotes are 70S while ribosomes of eukaryotes are 80S
  - E.g. streptomycin: binds to small subunit (30S) of bacterial ribosome such that initiator tRNA cannot bind to small subunit
  - E.g. tetracycline: blocks aminoacyl-tRNA from attaching to the A site of bacterial ribosome

- **Disrupting nucleic acid synthesis**
  - Enzymes used are different in conformation e.g. bacterial RNA polymerase ≠ human RNA polymerase
  - E.g. rifampin: inhibits RNA synthesis by binding to bacterial RNA polymerase ➔ preventing transcription

- **Administration of antibiotics**
  - Consumption at evenly spaced intervals: maintain a concentration of antibiotic in the body that is lethal to the bacteria (note: concentration will not increase as it is being metabolised as well)
  - Complete course of antibiotics: antibiotics will kill all susceptible strains, so that the immune system can focus on tackling the resistant strains (note: development of antibiotic resistance cannot be prevented, happens via spontaneous mutation)

- **Development of antibiotic resistance**
  - Failure to complete course of antibiotics ➔ some bacteria survive ➔spontaneous mutation in bacterial population produces antibiotic-resistant strains ➔ transfer of antibiotic-resistance genes from bacterium to bacterium via conjugation/ transduction/ transformation
  - When antibiotic is given, it acts as a selection pressure ➔ those with antibiotic-resistance gene survive, reproduce and pass the allele to daughter cells whereas those that are susceptible die ➔ over a few generations, microevolution occurs ➔ increased frequency of antibiotic-resistant allele in population
  - Examples of spontaneous mutations that can lead to resistance: mutations which led to the production of enzymes to break down antibiotics, or membrane proteins that inactivate/ pump out antibiotics

*** Treatment of Bacterial Infections- Use of Virus (Bacteriophage) vs. Antibiotics:**

- Virus / phage is very specific and will only attack a particular bacterial strain (vs. antibiotics which kill different types of bacteria, including useful ones)
- As bacteria evolve resistance, viruses can also evolve to overcome resistance (vs. bacteria evolve resistance to antibiotics over time)
- Viruses can replicate once they infect the bacteria
  - Only a small quantity of virus needed, as it can replicate once in the host to produce more viruses (vs. antibiotics are metabolised and eliminated from the body)
- Viruses stop reproducing once specific target bacteria are destroyed
- Less likely to cause side effects/ allergies to patient

*** Treatment of Viral Infections: Design of Drugs**

Target Aspect	Possible Drugs
Attachment	Drug that binds to specific host cell plasma membrane receptor ➔ prevents virus from binding and entering via endocytosis
Replication	Drug that carries antisense RNA that will bind to viral RNA to form dsRNA ➔ ribosome cannot bind ➔ translation cannot occur to replicate viral RNA
	Nucleoside analogs (resemble nucleosides): impair virus's ability to extend its RNA during replication as incorporating analog into RNA prevents addition of next nucleotide
Important virus-specific enzymes/ proteins	Drugs that inhibit RNA-dependent RNA polymerase (in influenza) ➔ prevents viral replication
	Drugs that inhibit viral reverse transcriptase/ integrase (in HIV) ➔ prevents integration of viral genome into host cell chromosomes ➔ prevents viral replication
	Drugs that inhibit viral protease (in HIV) ➔ cannot hydrolyse polyproteins into smaller functional protein components
	Drugs that bind to viral polyproteins (in HIV) ➔ cannot be cleaved by viral protease

*** Pathogens & Diseases:**

Disease	Influenza	AIDS (acquired immunodeficiency syndrome)	Tuberculosis
Pathogen	Influenza virus	Human Immunodeficiency Virus (HIV)	*Mycobacterium tuberculosis* • Obligate aerobe: requires oxygen for aerobic respiration, survival & growth • Peptidoglycan cell wall + mycotic acid layer (rich in lipids)
Target cells	Epithelial cells of respiratory tract: haemagglutinin on virus binds to sialic acid receptor found on epithelial cell membrane	T helper cells or macrophages of immune system: gp120 on virus binds to CD4 receptor on host cell surface membrane with the help of a co-receptor	Primary infection (pulmonary TB): lungs
Transmission	Droplets of moisture from lungs of infected person/ infected bird droppings	Primarily through unprotected sexual contact/ exposure to infected blood & blood products Can also be from mother to child via the placenta, during childbirth, or during breastfeeding	Airborne: transmitted in fine aerosol droplets (from ruptured tubercle) when an infected person with active TB sneezes/ coughs & an uninfected person inhales the droplets [*Spreads rapidly in overcrowded conditions]*
Pathogenicity	• Virus settles on mucous membrane lining the nose, pharynx, trachea, bronchi • Neuraminidase enzyme on surface of virus helps virus penetrate mucoproteins (glycoproteins) in mucus layer • Haemagglutinin binds to sialic acid receptors on cell membrane of epithelial cells lining respiratory tract ➔ virus penetrates into host cells & replicates within them • Incubation period: 24-48h, after which infected epithelial cells are destroyed ➔ leads to inflammation & build-up of dead epithelial cells in airways • Symptoms: runny nose, scratchy throat • Viral replication ➔ weakening of epithelial layer ➔ respiratory passage more susceptible to secondary bacterial infections, leading to fatal diseases like pneumonia **How viral infections lead to cell death** • Immune system recognises infected cells ➔ lysed • Budding off causes progressive loss of host plasma membrane ➔ cells eventually die • Host cell transcription and translation mechanism has been taken over by virus producing viral proteins, compromising vital functions of the cell ➔ cell death	• Once virus enters bloodstream, it targets T helper cells & macrophages: has a very strong affinity for & binds to CD4 receptors • Virus infects T helper cells: infected T helper cells destroyed ➔ T helper cell levels fall as more & more are destroyed • Virus infects macrophages: macrophages may survive HIV infection because they are not lysed by the virus ➔ act as reservoirs • HIV can pass from cell to cell in an individual, or to other individuals while remaining undetected • High rate of mutation of virus during replication ➔ altered proteins on surface of virus ➔ escapes recognition & elimination by immune system ➔ evolves rapidly within body • Increasing loss of T helper cells ➔ impaired immune responses ➔ increasingly susceptible to opportunistic diseases • Secondary infections e.g. tuberculosis ➔ death • Integration of viral dsDNA into host genome is random ➔ may result in the activation of a proto-oncogene to become an oncogene/ inactivation of a tumour suppressor gene ➔ development of cancer e.g. Karposi sarcoma is a rare skin cancer that is more prevalent in AIDS patients	• Once bacteria is inside lungs, alveolar macrophages phagocytose the bacteria • In the phagosome, bacteria inhibits fusion of phagosome with lysosome ➔ no phagolysosome formed, no lysosomal enzymes kill the bacteria • Bacteria survives, continues to multiply inside macrophage that phagocytosed them + more macrophages are brought to infected alveolus • A tubercle (ball-like clustering of cells like macrophages) is formed. At the centre of the tubercle, cell death by necrosis occurs ➔ involves rupturing of cell membrane & releasing of cell content (i.e. bacteria) (vs. apoptosis: shrinking) • Disease may be arrested at this stage & remain latent for years: persons who have TB infection, but not TB disease, do not spread disease to others (only sign is a positive reaction to tuberculin skin test/ TB blood test) • TB disease: tubercle ruptures ➔ bacteria spills into a bronchiole & spreads throughout the lungs ➔ productive cough that facilitates aerosol spread of bacteria • Rupturing of tubercles ➔ formation of cavities ➔ lungs progressively destroyed • TB often first opportunistic infection to strike HIV-positive people: if person has dormant TB, HIV likely to reactivate TB disease OR if person is previously uninfected, made more susceptible

Treatment	• **No treatment** for most people: **bed rest** & perhaps **aspirin/ paracetamol** to alleviate headaches & fever • **Antiviral drugs**: oseltamivir (Tamiflu) & zanamivir (Relenza) ➔ neuraminidase inhibitors: halt spread of virus in the body (suited towards Influenza A & B strains only) + amantadine & rimantiadine ➔ block a viral ion channel: prevents virus from infecting cells (Influenza A only) • **Antibiotics**: prevent secondary bacterial infection like pneumonia	• High rates of virus reproduction & mutation of virus ➔ generally **3 agents** administered in combination • Types of retroviral drugs include **reverse transcriptase inhibitors, protease inhibitors and integrase inhibitors** (inhibit respective enzymes) & **entry inhibitors** (block interaction between HIV envelope and CD4/ co-receptor, or prevent fusion of viral & host cell membranes ➔ block entry of HIV into cells) • **Sustained treatment** ➔ suppression of viral replication ➔ dramatically enhanced life-expectancy of infected individuals	• **6 months of daily treatment** with a combination of **at least 2 antibiotics**: combination used to minimise risk of developing resistance & achieve an additive effect against bacteria; skipping of a dose/ stopping before complete course increase chances of development of resistance ➔ potentially serious, difficult to treat, requires longer course • Antibiotic inhibits synthesis of mycobacterial cell wall e.g. antibiotic isionizid, or inhibits RNA synthesis during transcription e.g. antibiotic rifampin (interferes with prokaryotic RNA polymerase)
Prevention	Vaccination: contains purified & inactivated material from 3 common influenza viral strains		Vaccination: BCG vaccine prepared from live, attenuated *Mycobacterium bovis* (bovine TB); often used to prevent spread of TB among children