IMMUNOLOGY

(a) ***To use basic immunological principles to explain how the body defends against infection.***

(g) ***To describe passive and active immunity.***

(I) **<u>Overview of Immune System</u>**:

- The immune system is a multicomponent defence system that recognises and protects the host against pathogenic factors that can potentially damage tissues or organs
- These pathogenic factors include:
    - (i) Microbes – Bacteria, viruses, fungi, parasites, Etc.
    - (ii) Cells – Tumour cells, transplanted or transfused cells, viral-infected cells, Etc.
    - (iii) Macromolecules – Toxins, allergens, drugs, Etc.
- It has the ability to distinguish "self" and "non-self" tissues
- It is divided functionally into – (i) Innate immunity, and (ii) Acquired immunity

(II) **<u>Innate Immunity</u>**:

"Innate immunity" acts as the first line of defence

Characteristics of "innate immunity":

- (i) Natural immune mechanisms that exist from birth
- (ii) Act immediately (first line of defence)
- (iii) Response is non-specific, stereotypical and lacks memory

Comprises of three components:

(1) Physicochemical barriers → prevent pathogenic factors from gaining access to body
- (a) Epithelial barriers (skin, GIT, respiratory tract, genitourinary tract)
    - Secretion of "antibacterial substances" (Eg. lysozyme in saliva, gastric HCl)
    - High turnover of epithelial membranes
    - High flow rates of urine, saliva and tears
    - Acidic environment (especially low pH of stomach)
    - Normal flora (prevents growth of foreign organisms)
    - Mucous membranes secrete mucous that trap foreign particles and remove them via ciliary action
- (b) Innate reflex mechanisms (Eg. coughing, sneezing, vomiting, diarrhoea, Etc.)

(2) Humoral defences
- (a) Complements (see below)
- (b) Acute phase proteins – Plasma proteins produced by liver during acute inflammation/infection (Eg. CRP, Fibrinectin, α1AT, α2-macroglobulin) → aids in opsonisation, complement activation and regulation of the AIR
- (c) Proteolytic enzymes (Eg. lysozyme) – Break down bacterial cell wall → lysis

(3) Cellular defences
- (a) Granulocytes
    - (i) Neutrophils → main defence against bacteria and fungi as follows:
        - At site of infection, tissue damage releases mediators that activate circulating PMNLs and vascular endothelium and causes them to express adhesive proteins → results in "Margination" (PMNL to move along margin of endothelium) and "Diapedesis" (PMNL migrate through endothelium into tissues)

        - "Chemotaxis" – PMNL are drawn towards chemotactic factors (Eg. cytokines, complement, Etc.) in tissues
        - "Phagocytosis" – PMNL recognise foreign material (Eg. bacteria) using "surface receptors" (Eg. LPS) → leads to cellular ingestion. This process is enhanced by "Opsonisation", whereby the foreign material is coated with an "Opsonin" (Eg. IgG or C3b)
        - "Killing/Digestion" – Phagocytosed material is incorporated into a "Phagosome" and destroyed by an O_2-dependent process (via respiratory burst using H_2O_2, OH^- and O_2^- generated from O_2) and non-oxidative process (via lysosomal enzymes)
    - (ii) Eosinophils
        - Mainly present in tissues (esp epithelial surfaces) cf. circulation
        - Mainly implicated in allergic and parasitic responses → via release of "basic" granular material
    - (iii) Mast cells and Basophils
        - Basophils circulate in blood → become Mast cells when they enter tissue
        - Mainly implicated in allergic and parasitic responses → degranulate and release mediators (histamine, 5-HT, SRS-A, Etc.) when IgE binds to it
- (b) Monocytes and Macrophages
    - "Monocytes" enter blood from BM for 3 days before entering specific tissues (esp the reticuloendothelial system) → once in tissue, they can stay there for months-years as "Tissue Macrophage" (Eg. Liver (Kupffer Cells), Lung (Alveolar and Interstitial Macrophages), Bone (Osteoblast), CNS (Microglia), Kidney (Mesangial), LN and Spleen (sinus macrophages), Skin (Langerhans cells))
    - Actions of tissue macrophages
        - (i) Phagocytosis and killing/digestion of IC pathogens (Eg. Listeria), mycobacteria, parasites (Eg. Trypanosomes), and fungi
        - (ii) Ag presentation to T-cells
- (c) NK cells
    - Large granular lymphocytes (resemble lymphocytes morphologically but lack TCR, CD3 and Ag clonal specificity)
    - Actions:
        - (i) Lyse viral-infected cells, tumour cells, and allogenic (graft) cells:
            - Possess receptors that allow it to identify target cells – recognises (i) downregulation of MHC I (Eg. viral infected cell) and (ii) IgG bound to target cells
            - Causes cell lysis via – (i) Apoptosis (nucleus of target cell is rapidly fragmented by endonucleases), and (ii) Release of Perforin/Granzyme granules onto the target cells
        - (ii) Release of cytokines (IFN-γ and TNF) that activate phagocytes and recruit T-cells

(III) **<u>Acquired Immunity</u>**:

"Acquired immunity" is activated to produce a specific reaction against a pathogen when it breaches the "innate" immune system

Characteristics of "acquired immunity":
- (i) Specificity → immune system identifies a specific Ag on a pathogen where it then elicits an effector response to neutralise it
- (ii) Memory
    - 1° immune response – Initial exposure to Ag → delayed immune response generated by "virgin" immune cell activation following contact with Ag

    - 2° immune response – Subsequent exposure to Ag → population of "memory" immune cells generated following 1° immune response results in a more rapid, prolonged and powerful immune response
- (iii) Diversity → all possible reactions with different Ag randomly generated by genetic recombination and somatic mutations of a small number of germline genes
- (iv) Immunological tolerance → immune cells that recognise "self" antigen are eliminated

"Acquired immunity" is brought about by "Lymphocytes":
- (1) T-lymphocytes (80% of lymphocytes) → cell-mediated immune response
    - (a) Helper T-cell (66%)
        - T-cell receptor (TCR) and CD4 surface glycoprotein recognises "exogenous" Ag bound to MHC II on Ag-presenting cells (such as macrophages, monocytes, dendritic cells, B-cells)
        - Functions – (i) Release cytokines (IL-4, -5, -6, -10 and IFN-γ), (ii) recruit and activate macrophages and cytotoxic/suppressor T-cells, (iii) promote B-cell maturation into plasma cells/memory cells, (iv) promote Ab production by plasma cells, (v) type IV delayed hypersensitivity reaction
    - (ii) Cytotoxic T-cell (33%)
        - TCR and CD8 surface glycoprotein recognises "endogenous" Ag bound to MHC I on all nucleated cells
        - Functions – (i) Cell-mediated cytotoxicity (esp viral-infected cells, tumour cells, foreign tissue grafts) via release of enzymes (perforin, granzymes, caspases) that induce cell lysis and apoptosis, and (ii) Release of cytokines (INF-γ, IL-2, TNF, lymphotoxin)
    - (iii) Suppressor T-cell → Down-regulates immune response to maintain tolerance of self-Ag
    - (iv) Memory T-cell → Reside in lymphoid tissue and induces more rapid and powerful immune response with 2nd exposure to Ag
- (2) B-lymphocytes (20% of lymphocytes) → humoral immune response
    - Foreign Ag initiates a specific clone of B-cells to form "Plasma cells" and "Memory cells" in response to cytokines produced by Helper T-cells
    - "Plasma cells" – Produce and release large quantities of Ab's into circulation → Ab's agglutinate, activate complement, opsonise, or neutralise the foreign Ag
    - "Memory" B-cells – Reside in lymphoid tissue and induces more rapid and powerful immune response with 2nd exposure to Ag
    - B-cells can also act as APCs → present Ag to stimulate T-cell activation

Recognition of an antigen by the immune system is key to "Acquired immunity":

Definitions:
- "Antigen" is a substance that reacts with immune cell receptor (Eg. Ab):
    - It may or may not elicit an immune response
    - Types – (i) Proteins, (ii) Polysaccharides (Eg. LPS), (iii) Lipids
    - Ag's are generally HMW molecules (> 5 kDa)
- "Immunogen" is an antigen (generally must be "foreign" to the host) that elicits an immune response
- "Haptens" are LMW molecules (Eg. drugs) that are not immunogenic UNLESS coupled to a protein carrier (Eg. albumin)

- Immune system has 3 glycoprotein molecules that can bind Ag via reversible non-covalent forces (H-bonding, van der Waal's forces, electrostatic attraction). They are:
    - (1) Antibodies – Highest affinity for Ag
        - Produced by B-cells/plasma cells → possesses "Epitope" (recognition site for part of the Ag)
    - (2) T-cell receptors (TCRs) – Lowest affinity for Ag
        - Two types – (i) αβ TCR (90% T-cells) and (ii) γδ TCR (primitive T-cells)

        - TCR interacts with small Ag fragment derived by proteolysis from the original intact Ag → this Ag fragment is only recognised in association with MHC (Ie. cannot interact with soluble or free Ag) (See Below)
    - (3) Major Histocompatability Complex (MHC) – Middle affinity for Ag
        - Holds processed peptide Ag (either endogenous or exogenous depending on type of MHC molecule) within a cleft for interaction with the TCR of the appropriately MHC-restricted T-cell (See Below)

Immunoglobulin:

- Immunoglobulins are serum globulins with immune functions (Nb. NOT all Ig's have Ag-binding (or Ab) functions, but ALL Ab's are immunoglobulins!)
- Structure:
    - Composed of a 4 chain polypeptide structure – Two identical heavy chains (50-80 kDa) linked to two identical light chains (23 kDa) by an interchain disulphide bonds
    - Each light and heavy chain has – (i) Constant region (C-terminal end with constant a.a. sequence → effector function (Eg. complement activation)), and (ii) Variable region (N-terminal end with variable a.a. sequence → Ag binding site)
    - Within 4 chains → intra-chain disulphide bonds to form "Domains"
    - There is a "Hinge region" that is flexible:
        - Papain cuts above to produce – (i) 2 x F_{ab} (soluble; retains Ag-binding capacity), and (ii) 1 x F_c (crystallisable; retains effector function)
        - Pepsin cuts below towards the C-terminal to produce a divalent F_{ab2} (2 x Ag-binding sites with NO effector function)
- Diversity of Ig's:
    - Two types of light chains – $\varkappa$ and λ present in all Ig's
    - Five types of heavy chains – γ, α, μ, δ, ε produced through gene variation encoding the H-chain
- Function – Ig's bind reversibly via non-covalent forces to an Ag to have the following effect:
    - (1) Block receptor-ligand interactions (Eg. neutralize exotoxins)
    - (2) Mark Ag for removal via:
        - (a) Enhances opsonisation and phagolysis
        - (b) Activates Complement Cascade which can (i) Further enhance opsonisation, or (ii) activate complement-mediated lysis
        - (c) Lysis by Ab-dependent Cell Cytotoxic T-cells
- Types of Ig's:
    - IgG (75%; t½ 18-23 days)
        - Dominant Ig – Present everywhere (intra and extravascular), and can cross placenta
        - 4 subclasses (IgG1 – IgG4) → due to 4 different forms of γ heavy chains
        - Specific functions:
            - (i) Major antimicrobial activity in body
            - (ii) Late Ab activity (involved in 2° response)
            - (iii) Acts as an "Antitoxin"
            - (iv) IgG2: Opsonisation of bacterial polysaccharides (Ie. encapsulated bacteria)
            - (iv) IgG1 and 3: Activates Classical complement pathway
            - (v) Fc receptor – Signals for phagolysis by phagocytes, lysis by NK cells, and transfer from mother to foetus (protect newborn until immunocompetence develops)
    - IgA

        - Dimeric Ig with J-chain and Secretory component that is found in mucosa surfaces and secretions ONLY (GIT, respiratory tract, tears, saliva, breast milk) – Provides antimicrobial activity in those areas
    - IgM
        - Pentameric B-cell surface Ag receptor that is found in blood
        - Specific functions:
            - (i) Early Ab activity – Involved in 1° response
            - (ii) Good agglutinator – Has 10 bind sites with high AVIDITY (even though each site has low affinity)
            - (iii) Activates complement
            - (iv) Enhances opsonisation and phagolysis
    - IgD
        - Present with IgM on mature B-cells at very low [ ] → unknown function
    - IgE
        - Found mainly on epithelial surfaces (skin, GIT, respiratory tract); low [ ] in serum
        - Has high affinity for mast cell receptors – Binds to receptors to "sensitise" mast cells; with subsequent Ag binding to the receptor-bound IgE, produces mast cell degranulation
        - Function – Parasitic infections, atopy and type I hypersensitivity reactions

Major Histocompatibility Complex (MHC)/Human Leukocyte Antigen (HLA):

- MHC/HLA is a transmembrane dimeric glycoprotein present on ALL nucleated cells → presents processed Ag to T-lymphocytes
- Two types:
    - MHC I
        - Consists of α-heavy chain non-covalently linked to a β-2 Microglobulin light chain
        - Present on ALL nucleated cells (EXCEPT RBC & syncytiotrophs)
        - "Endogenously synthesised" Ag (Ie. viral proteins) are hydrolysed, processed by the ER and golgi apparatus into peptide fragments that are expressed with MHC I → presented to CD8+ Cytotoxic T-Cells
    - MHC II
        - Consists of an α-chain non-covalently linked to a β-chain
        - Present on APC's (esp macrophages, monocytes, B-cells)
        - "Exogenous" Ag are phagocytosed by APCs and hydrolysed/processed into peptide fragments that are expressed with MHC II → presented to CD4+ Helper T-Cells

Important to note – "MHC-Restriction" (Zinkernagel & Doherty):

- T-Cells exhibit MHC Restriction – T-Cells ONLY recognise foreign peptide associated with self-MHC that is of same specificity of the T-Cell (Ie. CD8+ T-Cells only recognise Ag bound to host-MHC I, and NOT host-MHC II nor foreign-MHC I)
- In the case of transplants/grafts, foreign MHC presented as foreign Ag with host-MHC!

(b) ***To identify effects of anaesthesia and critical illness on immune function.***

Effects of anaesthesia on immune function:

- Anaesthetic agents (and surgical stressors) cause reversible depression of immune function:
    - (i) Impairs physicochemical barriers
    - (ii) Decreased tracheal ciliary activity
    - (iii) Depressed phagocytosis and lymphocyte function due to hormonal changes (esp cortisol) associated with stress response
    - (iv) NK cell activity change in biphasic manner – Initial rapid and transient increase (due to recruitment from extravascular space, LN, spleen); then later on depressed by suppressor monocyte release

Effect of critical illness on immune function:

- Critical illness produces a stress response → ↑ cortisol, ↑ catecholamines (Adr, NAd), ↑ T3/T4, ↑ GH, ↑ glucagon
- Effects on immune function:
    - (1) Immunosuppression due to (i) ↓ immune cell proliferation, differentiation and activity (lymphocytes, monocytes, basophils, eosinophils, mast cell), (ii) ↓ cytokine levels, and (iii) ↓ complement levels
    - (2) Anti-inflammatory response due to (i) stabilisation of lysosomal membranes (↓ proteolytic enzyme release), (ii) ↓ capillary permeability (↓ histamine/bradykinin release, ↓ capillary leakage), and (iii) ↓ inflammatory mediators

(c) ***To explain the immunological basis and pathophysiological effects of hypersensitivity.***

Overview of "Hypersensitivity reactions":

- Defined as an exaggerated or inappropriate immune response that causes tissue damage
- There are 4 types (categorised by Gell and Coombs) based on:
    - (i) Ab involved
    - (ii) Nature of Ag
    - (iii) Type of cell mediating response
    - (iv) Duration of reaction

Type I: Immediate Anaphylactic Hypersensitivity

- Initial Ag exposure – Ag presented to T-helper cell → causes it to stimulate B-cells to produce specific antibodies (IgE) against the Ag → IgE then binds to mast cells via Fc receptor and sensitises them
- Re-exposure to Ag – Ag reaches sensitised mast cell → binds to and cross-links surface-bound IgE's on mast cell → causes mast cell degranulation in 2 phases:
    - (i) Immediate phase (15-30 mins post-Ag exposure) – Release pre-formed mediators (histamine, bradykinin, 5-HT, SRS-A, PAF) → cause ↑ vascular permeability (oedema), ↓ vascular SM tone (vasodilation), ↑ bronchial SM tone (bronchoconstriction) and ↑ mucous secretions
    - (ii) Late phase (6-12 hrs later) – Synthesis and release of mediators (esp SRS-A's, such as LTs and PGs) with progressive tissue influx of inflammatory cells (PMNL, monocytes, eosinophils)
- Clinical manifestation depend where and how Ag enters the body:
    - Local manifestations – Urticaria (hives), eczema, asthma, conjunctivitis (hay fever) → Ag contacts skin or respiratory mucous membranes in sensitised individuals
    - Systemic manifestations – Systemic anaphylaxis (hypotension/CVS collapse, bronchospasm, laryngeal oedema, skin rashes and death) → Ag administered parentally in sensitised individuals

Type II: Cytotoxic Hypersensitivity

- Antibody-mediated "cytotoxic" reaction where IgG and IgM Ab are directed at cell membrane surface Ag's (Eg. Ag source may be from pathogens or drugs stuck to membrane surface) → (i) activates complement via classical pathway, causing cell lysis, and (ii) induces Ab-dependent cell cytotoxicity
- Clinical examples – Organ-specific diseases (Eg. glomerulonephritis, myasthenia gravis), Autoimmune blood cell destruction (Eg. haemolytic anaemia, thrombocytopaenia), transfusion reactions, haemolytic conditions of newborn (Rh), hyperacute allograft rejection

Type III: Immune-Complex Hypersensitivity

- Immune complexes (or Ag-Ab complexes) are normally cleared by the reticulo-endothelial system → but when (i) there are too many complexes to clear or (ii) complexes are too small to be cleared effectively, these complexes deposit in tissues → elicits complement activation and PMNL infiltration → AIR and tissue damage
- Two types:
    - (i) Serum sickness (systemic form)
        - Serum Ag excess leads to immune complex formation in blood → complexes then deposit in tissues → cause systemic effects (Eg. fever, arthralgia, vasculitis, splenomegaly, lymphadenopathy)
        - Clinical example – Drugs or tetanus/diphtheria vaccine antitoxins
    - (ii) Arthus phenomenon (localised form)
        - Repeated exposure to an Ag results in production of a ↑↑↑ [ ] of serum IgG towards that Ag → re-exposure to the Ag leads to immune complex formation within the tissue site of Ag exposure → causes local effects

        - Clinical example – Inhalation of organisms in hay → Farmer's lung

Type IV: Delayed Type Hypersensitivity

- Initial Ag exposure – Ag is presented to T-cells by APCs → causes them to proliferate and form a sensitized population of CD4+ T-cells
- When Ag is represented to this sensitised CD4+ T-cell population by APCs → T-cells release cytokines (esp Il-2, Il-4 and IFN-γ) to cause:
    - (i) Activation of localised macrophages → kill microorganisms they contain
    - (ii) Attraction of lymphocytes and macrophages to the site of lesion
    - (iii) Fusion of activated tissue macrophages to form "Giant cells" (esp with prolonged Ag stimulation)
    - (iv) Granuloma formation (involves "Casseation" with fibrosis and calcification) → walls off infective focus
- Clinical example – Mycobacterium tuberculosis infection

(d) ***To outline the principles of management strategies of anaphylactic/anaphylactoid reactions.***

Overview of anaphylaxis:

- A life-threatening anaesthetic event involving a type I hypersensitivity reaction (IgE-mediated mast cell/basophil degranulation) that occurs in response to exposure of a triggering Ag in a patient who has been previously sensitised to the Ag
- Incidence 1:6000 to 1:20,000 – triggers include:
    - (i) Mainly NMBD (60%) – SCh > rocuronium > vecuronium > pancuronium > atracurium
    - (ii) Antibiotics (15%) – esp penicillin
    - (iii) Latex (15%)
    - (iv) Others (10%) – Gel-based colloids, LA, protamine, NSAID, contrast, Etc.

Mechanism of anaphylaxis:

- Initial Ag exposure – Ag presented to T-helper cell → causes it to stimulate B-cells to produce specific antibodies (IgE) against the Ag → IgE then binds to mast cells via Fc receptor and sensitises them
- Re-exposure to Ag – Ag reaches sensitised mast cell → binds to and cross-links surface-bound IgE's on mast cell → causes mast cell degranulation in 2 phases:
    - (i) Immediate phase (15-30 mins post-Ag exposure) – Release pre-formed mediators (histamine, bradykinin, 5-HT, SRS-A, PAF) → cause ↑ vascular permeability (oedema), ↓ vascular SM tone (vasodilation), ↑ bronchial SM tone (bronchoconstriction) and ↑ mucous secretions
    - (ii) Late phase (6-12 hrs later) – Synthesis and release of mediators (esp SRS-A's, such as LTs and PGs) with progressive tissue influx of inflammatory cells (PMNL, monocytes, eosinophils)

> Note – Anaphylaxis can occur without previous exposure to a triggering Ag → this is due to "cross-sensitisation" with Ag of similar structures in food, cosmetics, Etc.

Clinical features of anaphylaxis:

- Initial Ag exposure and sensitisation → no symptoms
- Upon re-exposure to Ag → Skin rashes, erythema, urticaria, abdominal pain/vomiting, laryngeal oedema (with AW compromise), bronchospasm, hypotension, tachycardia → profound CVS collapse → cardiac arrest and death

Management of anaphylaxis:

- (1) Cease administration of triggering agent
- (2) Call for help
- (3) Secure airway and ventilation
    - Consider securing AW (due to laryngeal oedema) with ETT if not done so → requires careful IV induction to avoid precipitating CVS collapse
    - 100% FiO_2
- (4) Adrenaline (most important therapy) → maintain C.O. (β1), ↑ BP (α1/β1), and ↓ bronchoconstriction/mucous secretions (β2)
    - IMI – 0.5-1 mg
    - IV – 1-10 ug/kg slow boluses (depending on severity of reaction) → repeated PRN (or requiring IV infusion), titrated to maintain CVS stability. 1 mg bolus every 2-3 mins with cardiac arrest
    - ETT or nebulised – 5 mL of 1:1000 for laryngeal oedema/bronchospasms
- (5) Liberal IVF resuscitation (crystalloid or colloid) → maintain C.O. and BP
- (6) Antihistamines – H1RB (promethazine 0.5-1 mg/kg IV) and H2RB (ranitidine 1 mg/kg IV) → antagonise systemic effects of histamine
- (7) Steroids – Hydrocortisone 2-6 mg/kg IV q6h → attenuate late inflammatory phase
- (8) Consider metaraminol or vasopressin infusion if resistant to adrenaline

- (9) Post-resuscitation – consider:
  - Serum tryptase → immediately and 1-3 hrs later to confirm anaphylactic reaction
  - ICU referral
  - Referral to allergy clinic 4-6 wks later for testing

Aside – Anaphylactoid reactions:

- Mechanism – Direct release of mast cell/basophil mediators by a triggering Ag
- Clinical features – Similar symptoms as anaphylaxis due to same mediators released by mast cells/basophils → clinically indistinguishable
- Differences between anaphylactoid and anaphylaxis reactions:

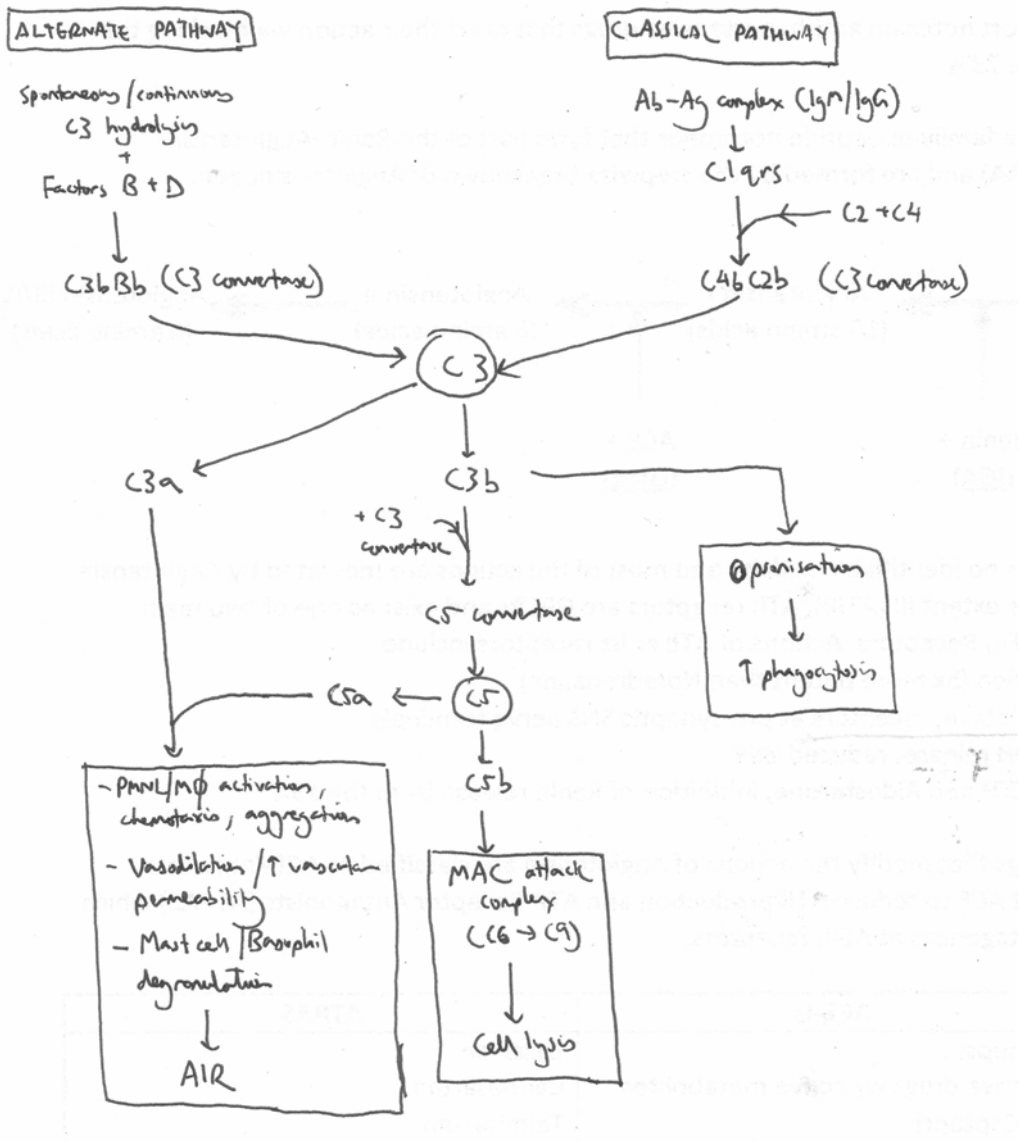
	Anaphylactoid	Anaphylaxis
Previous Ag exposure	Not required	Required
Reaction to Ag	Occurs with 1st exposure	Occurs with subsequent exposure
IgE mediated	No	Yes
Severity of reaction	Less severe	Severe and fatal
Response of reaction	Graded → reaction severity related to agent dose	All-or-nothing → reaction severity not related to agent dose

(e) ***To describe the role of complement.***

Overview of complement system:

- A proteolytic system present in plasma → part of innate immune system
- Consists a group of at least 25 serum proteins ("complements") produced 1°ly by hepatic parenchymal cells (but also macrophages at sites of inflammation)
- Complements are present in the circulation in an inactive form → activation occurs in a sequential manner with each activated component catalysing the activation of several molecules of the next component as part of an amplification response
- This process occurs via two separate pathways (Classical and Alternate), each with a different reaction sequence → they come together in final common pathway involving the breakdown of C3

Complement cascade pathways:



(1) Classical pathway:

- Trigger – Ag-Ab complex (involving only IgM and IgG) → Ab-DEPENDENT
- Process:
    - Complexing of Ag and Ab induces a conformational change in the Ab that exposes a binding site for C1
    - C1 has 3 components (C1q, C1r, C1s) – C1q binds to the exposed Ab site → converts C1r to an enzyme that activates C1s

- C1qrs-Ab-Ag complex cleaves C2 and C4 → forms C4b-C2a complex with "C3 Convertase" activity that cleaves C3 into C3b and C3a → final common pathway
- Nb. This pathway is controlled by several proteins (esp C1 esterase inhibitor) → minimises inappropriate activation of this complement pathway

(2) Alternative pathway:
- Trigger – Continuous and spontaneous series of reactions that only produces effects when pathogens and "Properdin" (insoluble polysaccharide) in the presence of C3b activate it → Ab-INDEPENDENT
- Process:
  - C3 spontaneously undergoes slow and continuous hydrolysis → hydrolysed C3 then binds Factor B and D → produces a complex with "C3 Convertase" activity that cleaves C3 into C3b and C3a
  - Some C3b formed binds Factor B and D → forms C3bBb complex that has more potent "C3 Convertase" activity → cleaves more C3 into C3b and C3a
  - C3bBb and C3b are rapidly cleaved and inactivated in blood → BUT the presence of pathogens and "Properdin" stabilises them and retards their inactivation → permits final common pathway to continue

(3) Final common pathway → both pathways lead to the formation of:
- (a) Activated C3b → which then:
  - (i) Complexes with "C3 convertase" (from either pathway) → forms "C5 convertase" which cleaves C5 into C5a and C5b → C5b binds to target cell membrane and then combines with C6, C7, C8 and C9 to form the "Membrane Attack Complex" → damages it and causes cell lysis
  - (ii) Acts as an opsonin – Coats target cells (Eg. bacteria) and provides site for phagocytes to bind via their C3b receptors → enhances phagocytosis
- (b) Activated C3a and C5a ("Anaphylatoxins" and "Chemotactic agents") → induce:
  - (i) Activation, chemotaxis and aggregation of PMNLs/monocytes
  - (ii) Vasodilation and ↑ blood vessel permeability
  - (iii) Mast cell/basophil degranulation → release active mediators (esp histamine)

<u>Clinical issues with complement</u>:
- (1) ABO blood group incompatibility:
  - Plasma Anti-A and Anti-B Ab are IgM → activate complement cascade via "classical pathway" when ABO incompatible blood is transfused → causes haemolysis of transfused RBC
- (2) C1 esterase inhibitor deficiency:
  - C1 esterase inhibitor is a protein that regulates several plasma protease systems (esp complement)
  - Deficiency of this protein is genetic (autosomal dominant) → triggers inappropriate complement activation → results in attacks of life-threatening angio-oedema (Ie. airway obstruction), usually triggered by minor trauma
  - Can be treated with Danazol → ↑ synthesis of normal C1 esterase inhibitor

(f) ***To describe the role of cytokines.***

Overview of cytokines:

- Low MWT proteins produced and released by certain cells in the body (not just immune cells) – (i) Lymphokines (by lymphocytes), (ii) Monokines (by monocytes), and (iii) Interleukins (by leukocytes)
- Provide intercellular communication to regulate – (i) Immune function, (ii) Inflammatory response, (iii) Growth and healing
- Made in small amounts and have short-lived BUT very potent effects

Mechanism of action:

- In response to cell surface signals (often other cytokines), cytokine gene is transcribed → low MWT protein (cytokine) produced and released
- Cytokine produces intercellular communication via (i) "Autocrine" (on same cell), (ii) "Paracrine" (on nearby cells) or (iii) "Endocrine" (on distant organs) means
- It acts on specific surface receptors of the target cell → modulates signal transduction events (Eg. protein phosphorylation) → produce effect in target cell
- Nb. "Pleomorphism" – A single cytokine can have several cellular targets and be produced by several cellular sources

Types of cytokines:

- (1) Interleukins
    - Inflammatory cytokines (Eg. Il-1 and -6 → fever, ↑ acute phase reactants, ↑ endothelial cell adhesion molecules)
    - Lymphocyte-derived mediators (Eg. Il-2, 9 → ↑ T-cell proliferation and differentiation; Il-4, 10, 13 → ↑ B-cell proliferation and differentiation; Il-5 → ↑ eosinophil production)
    - Macrophage-derived cytokines (Eg. Il-12 and -15 → ↑ T-cell proliferation, activity, and IFN-γ production)
- (2) Interferons
    - IFN α (leukocytes) and β (fibroblasts) → inhibit viral replication
    - IFN γ (T-cells and NK cells) → inhibit viral replication, ↑ MHC class I/II expression to promote Ag presentation, ↑ macrophages and CD8+ T-cell activity
- (3) Chemokines
    - Chemotactic cytokines that regulate immune cell migration (esp cells involved in inflammation/fibroblasts) into extravascular compartment
    - Each chemokine produced by certain cells attracts a specific cell (Ie. IL-8 attracts PMNLs, MCP attracts monocytes, RANTES attracts memory T-cells)
- (4) Tumour necrosis factors
    - TNF-α (macrophages/monocytes, T- and B-cells, NK cells, PMNLs, endothelial cells) and TNF-β (T- and B-cells)
    - Actions – (i) Upregulate adhesion molecules on endothelial cells → ↑ migration and activation of immune cells, (ii) ↑ MHC expression, and (iii) Induce macrophages to release cytokines, (iv) ↑ acute phase proteins
- (5) Growth factors:
    - Haemopoeitic Growth Factor (HGF) → Induce proliferation, differentiation and maturation of specific blood cells from pluripotential HSCs (Eg. GM-CSF)
    - Platelet-Derived Growth Factor (PDGF) → Stimulate mitosis of immune cells, phagocytosis, and secretion of proteinases
    - Transforming Growth Factor (TGF-β) → Stimulates humoral response and fibrosis by ECM formation

Nb. Differences b/t cytokines and hormones:

- Cytokines not produced by special glands (cf. hormones → specialised "ductless glands")
- Cytokines act mainly via paracrine and autocrine means (cf. hormones → endocrine)
- Cytokines are polypeptides/LMWT proteins (cf. hormones → not all polypeptides)

(g) ***To outline the principles of tissue/organ transplantation and the mechanisms of rejection of allogenic organs.***

The major issue with tissue transplantation is an "Allograft reaction" initiated the presence of transplanted cells. There are two types of such reaction:

(1) Host-versus-graft reaction (HVGR):
- HVGR → occurs when the host's immune system attacks and rejects the donor graft
- Mechanism:
    - Sensitization phase – Host immune system is exposed to foreign Ag (especially MHC) and is primed to attack the graft
    - Graft destruction is achieved by:
        - (i) Cellular response – Cytotoxic cells (CD8+ and NK cells) directly attack graft cells
        - (ii) Humoral response – Ab-induced complement lysis, phagolysis via opsonisation, Ab-dependent cell cytotoxicity
        - (iii) Lymphokines attract monocytes/macrophages to the graft tissue → attack it and release IL-1 to propagate the cellular response
        - (iv) TNF and IFN-γ → stimulate graft expression of foreign MHCs to attract more Ag recognition
- There are four types of HVGR:
    - (a) Hyperacute rejection (within minutes of transplant) – Interaction of preformed cytotoxic Ab in host's circulation with MHC I Ag on endothelial surfaces of graft → causes immediate complement activation, coagulation, microvascular thrombosis and graft infarction
    - (b) Accelerated rejection (within 4 days of transplant) – Host is previously sensitized against donor's Ag → rejection carried out by cellular and humoral immune system
    - (c) Acute rejection (within 1st month of transplant) – T-cell-mediated reaction
    - (d) Chronic rejection – Humoral immune reaction involving fibrosis and destruction of graft (due to chronic Ab-mediated destruction and arteriolar narrowing with graft ischaemia)

(2) Graft-versus-host reaction (GVHR):
- GVHR → occurs when immunologically competent graft cells attack the host's Ag (esp with BM transplant)
- There are two types of GVHR:
    - (a) Acute GVHR (within 4 weeks of transplant) – Causes dermatitis, jaundice, hepatosplenomegaly, and opportunistic infections
    - (b) Chronic GVHR (after 3 months of transplant) – causes hepatitis, pericarditis, myositis, and death from opportunistic infection

(h) ***To understand the principles of tissue typing.***

Tissue typing – Tests performed prior to transplantation to determine the MHC phenotypes (using HLA allele expression) of both donor and recipient → used to assess the compatibility of transplanted tissue and likelihood of graft rejection

Tests performed:

- (1) Serological methods – HLA-specific monoclonal Ab are used to phenotype MHC Ag on donor and recipient cells using immunofluorescence methods
- (2) Molecular typing – PCR fingerprinting, RFLP, sequence analysis, Etc. are used to type the MHC of donor and recipient
- (3) Mixed leukocyte reaction – Donor and recipient leukocytes are co-cultured for several days → degree of leukocyte proliferation (measured indirectly by uptake of radiolabelled DNA precursor) is proportional to MHC disparity b/t donor and recipient