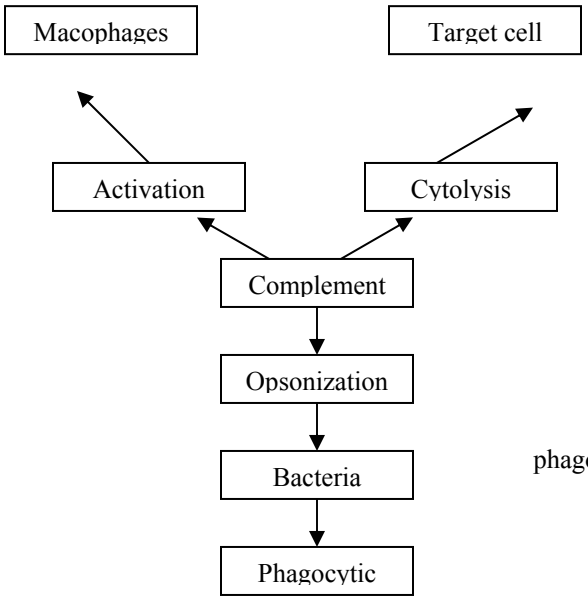
COMPLEMENT

- **Ability of Ab to inactivate foreign material depends upon collaboration of another factor, *complement*. Complement consists of a complex series of proteins many of which are proteinases.**
- **This system of enzymes non-specifically complements the immunological specific effects of Ab by the opsonization and lysis of red cells and bacteria.**
- **The complement system has 3 vital functions -Cell activation**
  - Cytolysis
  - Opsonization: redering cells vulnerable to phagocytosis by the adherence of opsonins e.g. complement component.



Complement facilitates the phagocytosis of antigen (e.g. bacteria)

Proteins of complement system form 2 interrelated enzyme cascades:

- **Classical pathway**
- **Alternative pathway**

- **These two routes bring about cleavage of C3, the central events in the complement system- a third set of plasma proteins which becomes assembled with the structures responsible for the lytic lesions in the lipid bilayer of foreign membrane, lethal to invading microorganism.**
- **Enzyme cascade: Generated by activation of *Enzyme precursors* which are fixed in turn to biological membranes. Each precursor is activated by the previous complement component or complex which is a highly specialized proteinase. This converts the *E precursor* to is catalytically active form by limited proteolysis → during which a small peptide fragment is released, a membrane binding site is exposed and the major fragment binds –so forming the next active complement *E* of the sequence.**

Since each enzyme can activate many *E precursor mol.* each step is amplified. The whole system forming an amplifying cascade resembling blood clotting.

THE CLASSICAL PATHWAY

Proteins of the Classical pathway

9 components known as complement 1-9.
Sequence of activation:C1, 4, 2, 3, 5, 6, 7, 8, 9.
- Most component are β-globulins: β_1 and β_2
- Large Mol. Wt ~ 2000 K
- Each component is made of 1 or 2 polypeptides joined by disulphide bonds.
- Except C4(3 peptides chains) and C1q(has a unique structure).
- One protein is a proteinase inhibitor which is a specific inhibitor of the serine protease $C1_s$ and $C1_r$.
- C3 is in largest amount (600 – 1800 mg/L) and fixation of complement in the major reaction of the complement sequence in molar terms.
- Majority of complement are synthesis by hepatic parenchymal cells, monocytes and macrophages.

Sequence of Events - 3 stages

- **Recognition**
- **Enzymatic activation.**
- **Membrane attack →cell death.**

Recognition – Fixation of C1 by Ig

- **C1 complex –3 subunits : C1q, $C1_r$, $C1_s$.**
- **C1 fixation occurs when the C1q sub component binds directly to Ig. $C1_r$ and C1s do not bind to the immunoglobulin but are involved in the subsequent classical pathway activation.**

Factors affecting C1 fixation

- **Only some subclasses of Ig can fix C1q (e.g. IgG1, IgG3, IgM in man)**
- **There are some special or configuration constrains (partially understood).**
- **One molecule of IgM fixes one C1q but 2 mol of IgG fix one C1q.**

The complement binding sites on Ig molecules:

The complement binding sites are on the CH2 domain of IgG and C_H4 domain of IgM.

- **The peptide sequence of the complement fixing site may become exposed following complexing of the Ig molecules, or;**

- **The sites may always be available but require multiple attachment by C1q with critical geometry to achieve the necessary avidity.**

C1q **– multivalent for attachment to the complement fixation sites of Igs.**

- C1q is a 400 κ protein

- It has 18 peptide chains in 3 subunits of six. Each 6 peptide subunit consist of a Y shaped pair of triple peptide helices joined at the stem and ending in a globular non-helical head.

-80 a.a. → Gly – X- Y where X and Y are pro, lle and OH lys - : resemble collagen fibrils.

- Globular ends are the sites for multivalent attachment to the complement fixing sites in immune complexed Ig. C1q fixes to the C_H2 domain of IgG and its subunits are held together as they are in plasma by a Ca^{++} ion acting as a ligand.

Sub units C3r and C1s:

- Chemically similar

- 83 Kd proteins

- C1r dimerises.

- C1s binds monovalently to C1r --forms a tetrameric complex which binds to C1q in the presence of a Ca^{++} ion.
- Activated by C1q (though this has no E activity).
- C1r and C1s activate in sequence still attached to C1q and both proteins can be inhibited by (Diisopropylfluorophosphate DFP) – and become serine histidine esterases on activation.
- C1r and C1s $\rightarrow$ a.a. structural homology and functional homology. Active site similar to that of trypsin and plasmin.
- C1r and C1s have different enzymatic specificity.
    - C1s in substrate for C1r
    - C1s activates C4 and C2 (but not C1r)

IgG +(C1q C1s C1r) $\longrightarrow$ (IgG – C1q C1s C1r)

Fluid phase of plasma

C4 $\rightarrow$ C4b

C4a (6 K peptide) from N terminal of α chain of C4

Fixation and activation of C4 and C2 by the C1qrs complex

- **In plasma "fluid phase".**
- **C1s splits a 6 K peptide (C4a) from N terminal of α-chain of C4 and activate it forming C4b – A labile reactive internal thioester bond in revealed on C4b.**
- **Binds weakly to membrane (~ 10%) close to the site activation, either to C1qrs complex or to adjacent red cell membrane.**
- **Free C4b → decay and are lost.**
- **C2 binds to C4b, forming a complex.**
- **C1s acts on it and cleaves a small C2a fragment (30 K) which is lost and C2b fragment (70 K) joins C4b to form a C4b2b enzyme, with catalytic site in C2b peptide.**
- **C4b2b -Unstable**

-decay – half life 5 min. at 37°C as C2b is lost and decays.

Inhibitor of C1

Protease inhibitor C1 esterase inhibitor (α2- neuraminoglycoprotein) which binds to C1s and C1r and inhibits them.

This is important in control of action of C4 and C1.

Membrane bond C4b is susceptible to decay by C3b inactivator.

Action of C4b2b complex on C3 to form C4b2b3b complex

C4b2b complex also known by 'classical pathway C3 convertase' activates C3 by splitting a 9 K peptide (C3 anaphylatoxin) from N-terminal end of the peptide of C3 and reveals a nascent thioester reactive binding site on the larger fragment C3b.

- C3b activated bind to membrane near C4b2b complex and some bind to it forming a C4b2b3b complex (proteolytic complex).

Action of C3b on C5

- C3b hydrolyses a 15 K peptide, C5a, from α-chain of C5 to initiate C5b fixation and the beginning of the *membrane attack complex*.

- No more proteinase are generated.
- C3b mol bind to the membrane that do not form C4b2b3b complex for an opsonic macro molecular coat on the red cell or other target particles and render it susceptible to immune adherence by C4b receptors on phagocytic cells. This is a major biological function of complement.
- C4b receptors on Neutrophils, Eosinophils, Monocytes, Macrophages (Kupffer cells, Alveolar macrophages)

- C3b – coated particles adhere to the phagocytic cell membrane and cause phagocytosis. Bacteria of low virulence may be phagocytosed with C3b alone. C4b coating acts similarly, but are less effective

C3b (C4b) coating

-Facilitates adherence of bacteria, viruses and neutrophils to monocytes and macrophages.

-Facilitates ingestion of certain bacteria by neutrophils and monocytes.

-Facilitates ingestion by activated macrophage.

-Facilitates IgG induced phagocytosis and IgG mediated cell cytotoxicity (ADCc).

Assembly of the C5-9 membrane attack complex

Fixation of C5b to biological membrane is followed by the sequential addition of four more proteins C6, C7, C8, C9 – In molar ratio these form the membrane attack complex.

C5b – C6 complex → hydrophilic

C7

C5b – C6C7 → Exposure of a polar gps which have detergent and phospholipid binding properties.

→ In free solution the C5b67 complex has half life of 0.1 sec

C8

and attacks any lipid bilayer → causing 'reactive lysis'

→ Membrane bond C5b67 is fairly stable and interacts with C8 and C9.

→ It is amphiphilic and palorizes to form small protein micelles

C5b678 Complex

C9

C5b6789 (membrane attack complex – a tubule traversing the membrane. Highly amphiphilic – like a cylinder projects from both sides of membrane).

- Allow free exchange of electrolytes and water across the membrane.

  Net influence of Na^+ and water → lysis

  Viral envelope membrane formation

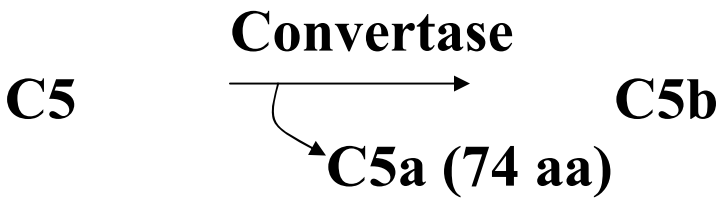
  Viral destruction

C3a and C5a

C3 —Convertase→ C3b

C3a (77 aa)

(from N-terminal) of α-chain)



- **In both cases a terminal Arg is revealed.**
- **Both C3a and C5a have 'spasmogenic properties' due to C-terminal Arg.**

C3a

- **Causes smooth muscle contraction**
- **Causes erythema and oedema in skin (due to histamine release in some cases)**

- Carboxypeptides-- C3b + Arg (inactive)

C5b

-10 times more effective than C3b

-Less conc.

-Causes blood neutropenia as it is a major chemotactic factor and causes neutrophil migration in vessels.

-Activates neutrophil.
-Switches on neutrophil production of leukotriens particularly B4 which prolongs the ↑ permeability phase induced by C5a.
-Causes neutrophil activation
-Increase vascular permeability
-Causes mast cell degranulation
-Causes smooth muscle contraction
-When treated with carboxypeptidase Arg is lost and C5a loses activity.

The Alternate Pathway

-This pathway has no antibody involvement.
- C3 is converted to C3b possibly by proteolytic E in the body fluids
- Certain surface e.g. polysaccharides (activator surface) favour the uptake of factor B on to the C3b.
-Factor D converts this to C3bBb (C3 convertase) which converts C3 to C3b.
- Factor H competes for the same binding site as B and C3bH is inactivated.
[in Paroxysmal nocturnal haemoglobunuria (PNH) the patients red cells act as activator surface leading to complement fixation by

alternate pathway-----lysis of red cells---blood in urine.]

- Binding of properdin (P) extends the half life of C3bBb forming C3bBb P, the half life in extended to 30 min.

Properdin levels are activated in some diseases, thus causing complement activation by this mechanism.

<u>Breakdown of C3b</u>

- **Free C3b or C3b fixed to a non-activator surface is susceptible to factor H binding.**
- **Structure in altered and it is cleaved by C3b inactivator (KAF, C3b1NA and now factor 1).**

  α- chain $\rightarrow$ 68 K + 43 K + 3 K
  (117 K)
- **C3b loses its haemolytic activity and immune adherence activity.**
  Further breakage leaves 29 K (C3d) on the red cell. Remainder C3c is removed by REC.

Effect of C3-9 depletion on the adaptive immune response

C3 – C9 have a function in the generation and functioning of memory cells after primary I.V. immunization (with thymus dependent antigen).

- **Def. of complement**
  - **mostly autosomal**
  - **Total absence of complement protein → due to absence of functional structural genes.**

i.C def. → immune complex like or lupus like disorders (due to failure of complement-dependent mechanism to eliminate immune complexes).

(glomerulonephritis; arthralgic, vasculitis)

ii.Absence of C3 → severe life threatening infections (pneumoncoccal, menningococcal, septicaemia).

iii.↑ association of C5, C6, C7 and C8 def. → with disseminated menningococcal and gronococcal infections

-Rheumatoid syndrome

iv. C9 def. - None

Anaphylatoxins

- Low
- Induces degradation of mast cells. and/or basophil.
  Causes release of various substances (Histamine)
  - ------ permeability
  - Causes contrative of smooth moles.

Anaphylaxis and other allergic reaction.

C3a and D5a → Anaphylatoxins.
Also C4a
Local edema

Chromatoxins Attract phagocytic cells.
Causes their ingestion (chromatosis)
C5a.

Immune Adherence C3b → immune adherence.
Particulate Ag, coated with Ab in pursuance of adhere to various surface.

Opsouization (C1b) Opsoui)

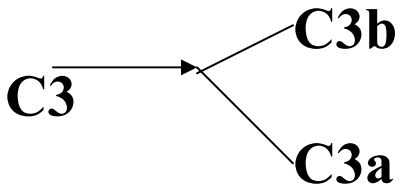
Attracted by phagocylic cells

Phagocytosis

Calssified pathway

The antibody focuses the activation of the components of complement on analgin.

C142



Release of various physiologically activesubstances.

Alternative Pathway

- **Does not need antigen Ab complex.**
- **Triggered by lipopolysarrcherides (LPS)**
  - **or edotoxis from the cell wass of Cm $^{-ve}$ bacteria.**
- **by the cell walls of some bacteria.**
- **by the cell walls of some yeasts (C3-------)**
- **by aggregated IgA.**
- **Factor in lobravenous.**

Ab – As

↓

Classical C1qrs

↓

C14

↓

C142: C3 connection.

↓

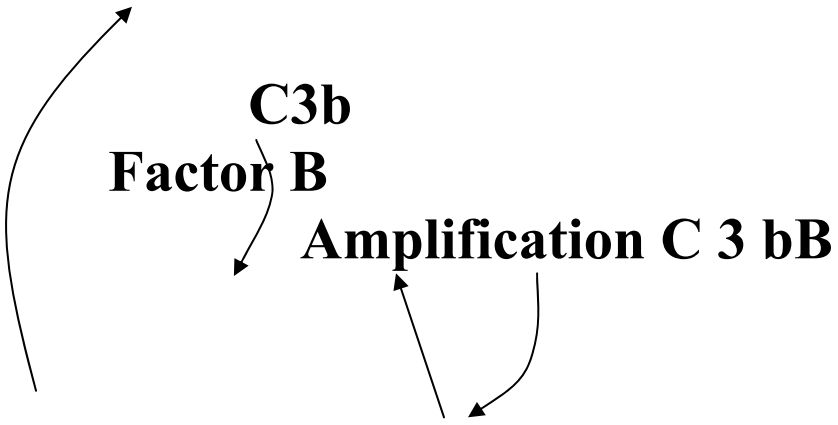
C3 → C3 a (promoters immune adhesion (between bacteria and macrophages ↑ phagocytes)

Exists in trace almost in normal serine

C3b → C3 5 6 7 8 9 (motivated by factors H and factor I (C3b inactivation)

Alternative pathway

Serum factor B



Activation by factor D cleaves

C3bB ← factor B attached to C3b.

Factor D → C3bBb (Enzyme complex)

(C3 –convertase)

Cobro vein

(Dissociations rapidly)

cell wall of gm $^{-ve}$ bacteria,

Stabilized by properdin

some yeast IgA aggregen

(serum protein)

CELLS INVOLVED IN THE IMMUNE RESPONSE

Vertebrates

Several organs and drift types of cells recognize non-self antigens on Mos and to eliminate them

Lower Animals: more primitive of proteins and phagocytes.
Vertebrates have lymphoid cell and lymphoid organs.
Phagocytes = Important defense in all animals.

All cells of immune system derived from pluripotein stem cell through 2 lines of differentiation.

The lymphoid linage → produce lymphocytes

- **T**
- **B**

The myeloid linage → produce phagocytes and other cells.

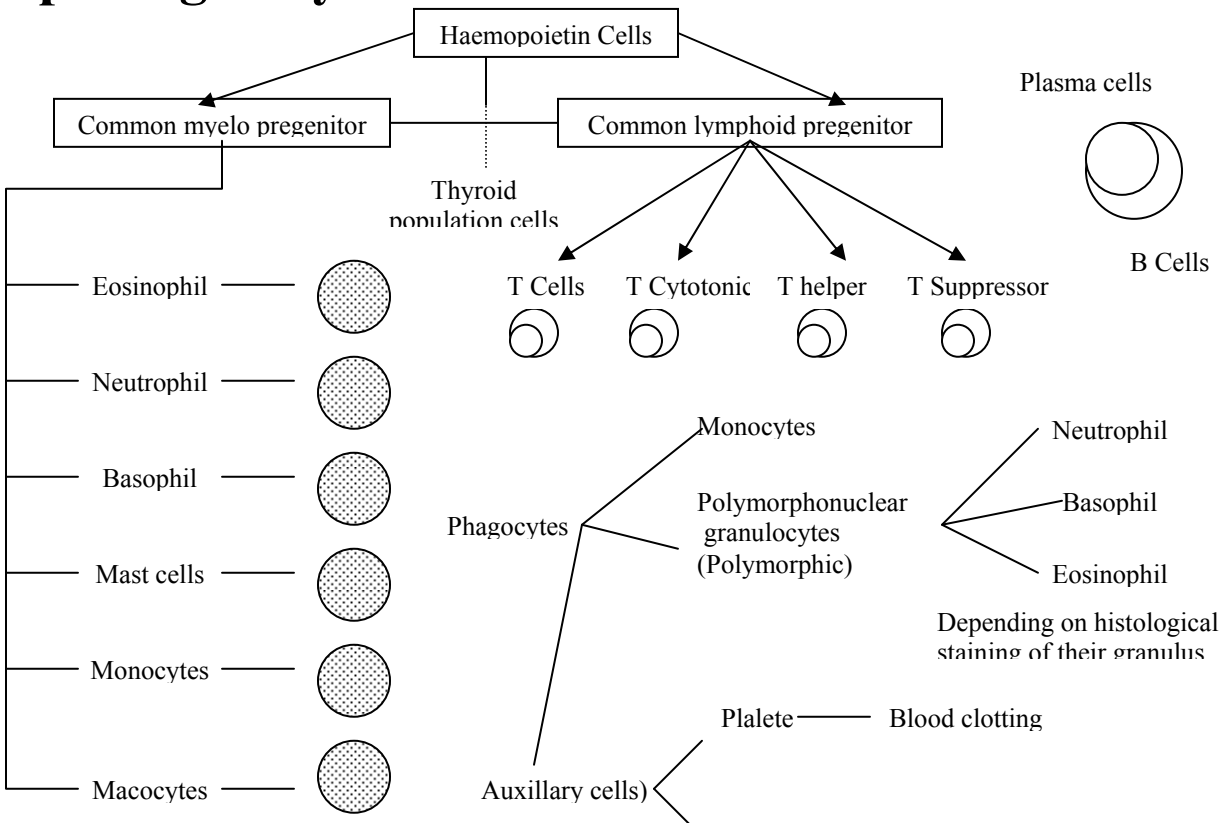
Lymphocytes

T-cells – Differentiate in thymus
B-Cells – differentiate in foetal liver spleen, and in adult bone marrow. In birds → in a organ known as bursa of Fabricius.
Null cells → Differentiation sequence (Non T, Non B cells) uncertain (Have intracytoplasmin granumles)

- **Have Fe receptors**

- **Pub. of bone marrow origin.**

All cells functionally distinct. But T&B cells morphologically identical



<u>Lipid Cells:</u> **Produced in pri**

lymphoid organs (Thymus, adult bone marrow)

- **High rate 10^9/day**

Spleen

- **Some migrate to a blood to secondary lymphoid tissues Lympj node**

tonsils, adrenal

Unencapsu

lated

lymphoid tissue

In Adults

- **Lymphoid tissue: ~2% of body weight.**
- **~10^{12} lymphoid cells - ~20% of total WBC (hameolyte, major WBC are PMN)**
- **Mature lymphoid cells → live long.**
- **May persist as memory cells**
- **Heterogeneous in size (6-10 μm diameter) and morphology.**

Different nuclear cytoplasm ratio
Different in degrees of cytoplasm staining with histological dyes.
Presence of absence of azurophilic granules.

T Cell

Typical small lymphocyte – Agranalar

B Cell

↑ nuclear: cyto ratio

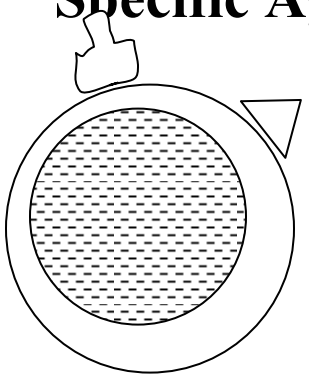
Azurophilic granules

Large lymphocyte (LGL)

Nuclear: Cytoplasmic ratio

T-Cells

- **Small lymphocyte**
- **Appear similar**
- **Have different cell surface antigen – Markers**
- **Most express 3 surface glucoproteins detected by monoclomal antibodies**
  T11, T1, T3
  (Mol. Wt. 55Kd) (67Kd) (20 KD)
- **Some markers occur only on cells subpopulations only i.e. on Thelper cell, etc.**
- **Surface antigens as receptors for the Fc region of AS found on both T- and B-cells. May play a role in regulation of lymphocyte.**
- **Responses have some specific acid hydrolysen.**

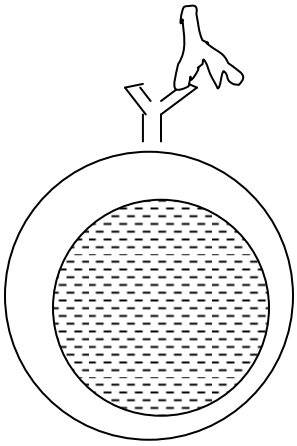


Cell surface markers on mouse and human peripheral B-cell

<u>B-Cells</u> - 5-15% of circulating lymphoid pool.

Characterized by endogenously produced Abs.

- **some inserted in membrane – Act as specific antigen receptors.**
- **Can be detected by with fluorescent labeled, specific antibodies.**
- **Majority express IgM and IgD –v-few express surface IgG, IgD and IgE. Though such cells present in specific location e.g. IgA-bearing cells in the gut.**



Fluorescent anti-body

Antibody

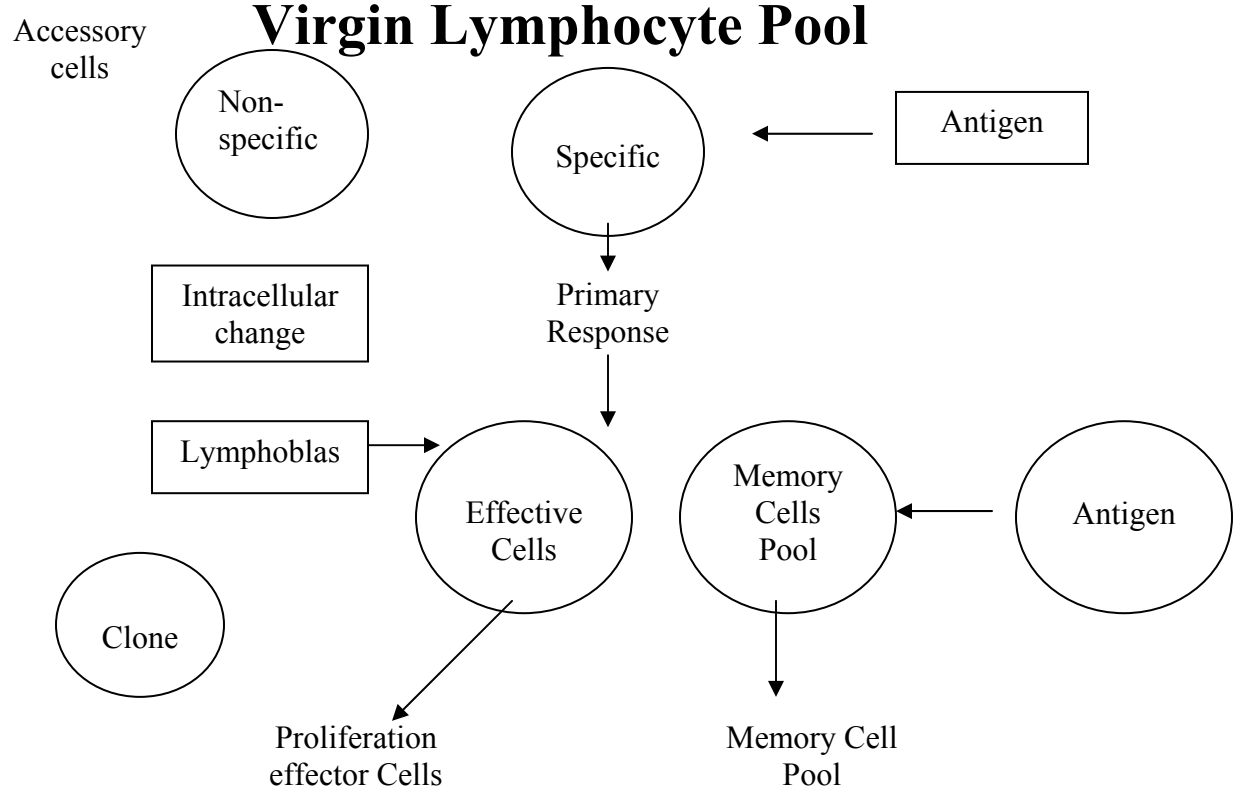
- **Divalent Abs crossline antigens – surface membrane glycoprotein – seen as 'patches' of crosslinked Ag-Ab complexes**

on cell surface. Most complexes swept along the cell surface – seen as 'caps' over one pole or cells.

- **Many other markers on B cells**

<u>Polymphocyte proliferation and maturation</u>

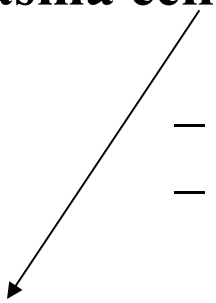
During development both T and B lymphocytes acquire specific receptors for antigens → which commit them to a single antigenic specificity for the rest of their life-span.



- **T & B cells are activated by different mitogens.**

- **Mitogenic lectins (protein that bind and cross-linke specific cell surface CHO determinants) will polyclonally stimulate lymphoid cells.**
- **These mitogens lectins (Mitogenes) are derived from various plants and bacteria).**

- **Ultimately B cells → differentiate to plasma cells.**



(< 0.1% of lymphocytes)
- **– not in circulation**
- **– Restricted to secondary lymphoid organs tissues.**

Produce antibodies of one specificity and Ig class.

<u>'Null' or "Third population Cells"</u>

- **Lymphocytes, but not T or B cell.**
- **Do not have markers of T or B cells**
- **Have Fc receptors for IgG – characterized.**
- **Probably of bone marrow origin.**
- **Look like – large granular lymphocyte.**
- **May contain majority of natural killer and antibody dependent cellular cytotoxic effectors (ADCC).**
- **NK cell → non-specificity kill tumour cells and virally infected cells and play a role in regulating immune response. Kill non specifically but its antibodies bond to their target cells.**

Mononuclear phagocyte system (Monocytes)

- **Derived from myloid preginator.**
- **2 man function**
  a. **Phagocyte macrophages whose predominant role is to remove particulate antigens.**
  b. **Antigen – presenting cell whose role is to present antigens to specific antigen – sensitive lymphocyte.**

Reticuloendothelial system

The phagocytic tissue macrophages form a network with RES – found in many organs. The cells of this system include:

- **Circulating blood monocyte.**
- **Kupffer cells in the liner.**
- **Enobthelium fixed – Intraglomerular mesangium of the kidney.**
- **Wandering macophage:**
  - **Alveolar macrophages in the lung.**
  - **Serosal macrophages.**
  - **Brain microglia**
  - **Spleen sinus macrophages.**
  - **Lymph node sinus macrophages.**

Derived from pro-monocyte in bone marrow

Circulating blood monocytes — **Represent circulating pool**

Migrate to various organ and tissue and become macro phages

Studied genetic detail

- **May live for months or years.**
- **Large cell (10-18 μm diameter)**
- **Have horse shoe shaped nucleus**
- **Often contain faint azurophilic granules.**
- **Have many intramacroplasmic lysosomes and well developed Golgi apparatus.**
- **Have hydrolysone and peroxidases for intracellular killing of Mos.**
- **Adhere strongly to glass and plate surface.**
- **Actively phagocytes organism and tumour cells.**
- **Bind to Mos by special receptors for IgG (Fc^{γ} receptor) and complement (e.g. Gb) which the Mos is coated.**
- **Carry other surface markers**

- **Also have receptors for lymphokines e.g. γ-1 interferon and migration inhibition factor.**
- **Functions enhanced by factors released from T cells.**
- **Also produce complement compounds, postgrlandins, interferons and monokines (e.g. Interleukin 1).**

<u>Antigen Presenting Cells</u> (APC)

- **Found in** **Skin e.g. Langerhan's cells**
  Lymph nodes (with Birbeck granule)
  Spleen

Thymus (crucial in development and maturation of T cell

- **Man role in to present antigen to antigen sensitive lymphoid cell.**
- **Rich in class 2 MHC antigens which are important for presenting antigen to T cells.**

<u>The polymorphs (PMN Granulocytes)</u>

- **Contain a multi-lobed nucleus and many granules.**
- **Produced in bone marrow.**
- **Rate 80 million/min.**

- **Short-lived (2-3 days)**
- **Granulocyte → 6-70% of total normal blood leukocytes.**
- **Able to adhere to and penetrate the endothelial cells lining the blood vessels.**

Classified as → **Neutrophils**, **Eosinophils**, **Basophil**

- **Do not show specificity for any antigen.**
- **Play an important role in acute inflammation and protect against Mos.**
- **Function → Phagocytosis.**
  ↓ No. of polymorphs and ↑ in susceptibility to infection.

<u>Neutrophils</u> **~90% of circulating granulocyte**

-10-20 μm in diamter.

- **Have 2 types of granules Primary (azurophilic) granules (lysome) contain hydrolase, peroxidase and lysozyme**

- **Contain the injected organism in vacuoles called phagosomes which fuse with the enzyme containing granule to form the phagolysome.**

<u>Eosinophils</u> **- 2.5% of blood leukocytes in non-allergic.**

- **Phagocytosis and killing Mos.**

- **Granules are membrane-bond organelles with 'crystalloid' or 'core'.**
- **Have biloded nucleus and many cytoplasms, metabolically active.**
- **May be degranulated → fusion of intracellular granules with plasma membrane → release pf content to the outside of cell**
- **In use granules armment against large targets which cannot be phagocytosed.**
- **Special role in immunity to helmiuth (protozos and worms) infection.**
- **Attracted by products released from T-cells, mast cells, basophils.**
- **Release histaminase and aryl sulphatase which unactivates the most cell products**

histamine and slow reactive substances of anaphylaxes.

↓ the inflammatory response and ↓ granulocyte migration into the site of invasion.

Basophils and Mast Cells

- **Very few No. (<0.2% of leukocyte)**
- **Have deep violet blue granules**
- **Mast cell may not be distinguished from basophils.**

- **Of bone marrow origin.**
- **Have randomly distributed granules surrounded by and containing membrane.**
- **Granules contain heparsin in both B&MC, SRS-A and ECF A.**
- **These are released on degranulation under appropriate stimuli – e.g. a allergen which cross-links specific IgE mol bond to the surface of the mast cell or basophil via Fc receptor for IgE.**
- **↑ release → symptoms of allergy.**
- **May play a role against paracytes.**

Platelets

- **Role in clotting**

- **Also in immune response esp. inflammation.**

Possess Class I MHC products and receptors for both IgG and