Blood coagulation
Hemostasis

- Arrest of bleeding
- Events preventing excessive blood loss
  - Vascular spasm
  - Platelet plug formation
  - Coagulation or blood clotting (secondary hemostasis)

Primary hemostasis
Steps in Primary Hemostasis

- **Vascular Constriction:**

  *Immediate constriction* of blood vessel

  Vessel walls pressed together – become “sticky”/adherent to each other

  Minimize blood loss
**Platelet Plug formation:**

PLATELETS attach to exposed collagen with the presence of von Willebrand factor (vWF) and Glycoprotein IbIX

Aggregation of platelets causes release of chemical mediators *(ADP, Serotonin, Thromboxane A$_2$)*

ADP attracts more platelets

Thromboxane A$_2$ (powerful vasoconstrictor)

* promotes aggregation & more ADP

**Leads to formation of platelet plug!**
Primary hemostasis: Vasoconstriction & Plug Formation

1. Exposed collagen binds and activates platelets.
2. Release of platelet factors
3. Attracts more platelets
4. Aggregate into platelet plug
Steps in Secondary Hemostasis

- Blood Coagulation (clot formation):
  
  **Final Step in Hemostasis:**
  
  - Transformation of blood from liquid to solid
  - Clot reinforces the plug
  - Multiple cascade steps in clot formation
  - Process requires plasma proteins, PLs and calcium.
  - **Soluble fibrinogen** \(\xrightarrow{\text{Thrombin}}\) **Insoluble fibrin**
Stages of Coagulation

- Activation of prothrombinase
- Conversion of prothrombin to thrombin
- Conversion of fibrinogen to fibrin

Pathways

- Extrinsic
- Intrinsic

Initially independent, then they converge on common pathway leading to the formation of a fibrin clot!
Secondary Homeostasis: Clot Formation

Stage 1 can be activated in two ways:

Extrinsic clotting pathway starts with tissue factor, which is released outside of the plasma in damaged tissue.

Intrinsic clotting pathway starts when inactive factor XII, which is in the plasma, is activated by coming into contact with a damaged blood vessel.

Stage 1: Damage to tissue or blood vessels activates clotting factors that activate other clotting factors, which leads to the production of prothrombinase. The activated factors are within white ovals, whereas the inactive precursors are shown as yellow ovals.

Stage 2: Prothrombin is activated by prothrombinase to form thrombin.

Stage 3: Fibrinogen is activated by thrombin to form fibrin, which forms the clot.
Hemostatic Clot Formation

**PRIMARY AGGREGATION**

Platelet Aggregation  →  Clotting

**SECONDARY COAGULATION**

Thrombin  →  Fibrin

Hemostatic clot

0 min  →  5 min  →  10 min
A **cascade** is a mechanism in which enzymes activate other enzymes sequentially usually leading to an amplification of an initial signal.

Each of these pathways leads to the conversion of **factor X** (inactive) to **factor Xa** (active)
Coagulation Factors

- The **intrinsic and extrinsic** coagulation pathways are a series of reactions involve coagulation factors known as
  1. Enzyme precursors (**zymogens**)  
  2. Non-enzymatic (**cofactors**)  
  3. Calcium (**Ca^{++}**)  
  4. Phospholipids (**PL**)  

- All coagulation factors normally are present in the plasma, with **PL** being provided by **platelets**.
Coagulation Factors

- **Zymogens:**
  - Factors II, VII, IX, X, XI, XII, and prekallikrein
  - **NO** biologic activity until converted by enzymes to active enzymes called **serine proteases**

- **Cofactors**
  - Factors V, VIII, tissue factor, and HMWK
What Triggers Intrinsic/Extrinsic Clotting?

- **Extrinsic**—Release of biochemicals from broken blood vessels/damaged tissue.

- **Intrinsic**—No tissue damage, blood contacts damaged endothelial layer of blood vessel walls.
Intrinsic vs. Extrinsic Clotting

- **Intrinsic clotting**—all factors are found in circulating blood.

- **Extrinsic clotting**—Factor III (tissue thromboplastin) is found outside of blood.
Intrinsic Pathway

- The formation of clot in response to abnormal vessel wall in absence of tissue injury is the result of intrinsic pathway.

- Begins with the activation of factor XII (Hageman factor).
Intrinsic Clotting Pathway

- **Under normal physiological conditions**, it is less significant to hemostasis than extrinsic pathway

- **Under abnormal physiology** (hyperlipidemic states; bacterial infiltration) activation of thrombosis via intrinsic clotting cascade
Intrinsic Clotting Pathway (cont...)

- The intrinsic pathway requires:
  1. **The factors** VIII, IX, X, XI, and XII
  2. **The proteins**: Prokallikrein (PK), High MW Kininogen (HK)
  3. **Calcium ions**
  4. **PLs** from platelets
A foreign surface such as collagen activates factor XII (Hageman factor).

Acting as catalysts are high MW Kininogen (HMWK) and kallikrein in the contact phase.
Initiation of the intrinsic pathway occurs when Prokallikrein (PK), high MW Kininogen (HK), factor XI, and factor XII are exposed to a negatively charged surface — this is termed contact phase.

Contact phase occurred as result of interaction with:

- PLs,
- Circulating lipoprotein particles (VLDL, Chylomicrons...)
- On the surface of bacteria
Contact Group

- XI, XII, HMWK, PK
- Not Vitamin K dependent
- The contact group is adsorbed by contact with a negatively charged surface such as collagen or the subendothelium in vivo.
Intrinsic Pathway  
(Contact Activation pathway)

- Calcium is involved in three steps: the activation of FIX, X and FXI.

- Cofactor VIII interacts in the activation of factor X and cofactor V reacts with prothrombin.

- The platelet PL surface acts as template in the activation of FX and prothrombin.
Extrinsic Pathway (Tissue Factor pathway)

- Is initiated by the release of tissue thromboplastin (Factor III) which is exposed to the blood when there is damage to the blood vessel.

- **Factor VII** which is a circulation coagulation factor, forms a complex with tissue thromboplastin and Ca2+.

- This complex rapidly converts **Factor X** to the enzyme form **Factor Xa**
**Common Pathway**

- **Factor Xa** catalyzes the prothrombin (Factor II) to thrombin (Factor IIa) reaction which is needed to convert fibrinogen (Factor I) to fibrin.

- XIIIa and Ca++ stabilize fibrin clot

- Formation of blood clot causes more clotting to occur—positive feedback.
Prothrombin is a soluble single chain glycoprotein (72kDa) synthesized in the liver.

Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated Factor X (Xa) and generate active **2 chain thrombin molecule** which is then released from the platelet surface.

The A and B chains of thrombin are held together by a **dissulfide bond**.

Prothrombin (72kDa) converts fibrinogen to fibrin.

Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated Factor X (Xa) and generate active 2 chain thrombin molecule which is then released from the platelet surface.

The A and B chains of thrombin are held together by a dissulfide bond.

Prothrombin (72kDa) converts fibrinogen to fibrin.
The activation of prothrombin occurs on the surface of activated platelets and requires assembly of prothrombinase complex consisting of platelet, anionic PLs, Ca\(^{2+}\), factor Xa and prothrombin.

This complex is termed factor Va which is activated by traces of thrombin.

Factor Va is subsequently inactivated by further action of thrombin to limit activation of prothrombin to thrombin.
340kDa (factor I) is soluble plasma glycoprotein that consists of 3 non identical pairs of polypeptides chains (Aα, Bβ, γ) covalently linked by disulfide bonds. The A and B portions of the Aα and Bβ chains, termed Fibrinopeptide A (FPA) and Fibrinopeptide B (FPB).

Release of FPs by thrombin generate fibrin monomer (weak).

Aggregate spontaneously forming insoluble fibrin polymer (fibrin clot) (hard, insoluble).
<table>
<thead>
<tr>
<th>Factor</th>
<th>Name</th>
<th>Function</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>Converted to fibrin</td>
<td>Common</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>Converted to thrombin (enzyme)</td>
<td>Common</td>
</tr>
<tr>
<td>III</td>
<td>Tissue thromboplastin</td>
<td>Cofactor</td>
<td>Extrinsic</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium ions (Ca^{2+})</td>
<td>Cofactor</td>
<td>Intrinsic, extrinsic, and common</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin</td>
<td>Cofactor</td>
<td>Common</td>
</tr>
<tr>
<td>VII*</td>
<td>Proconvertin</td>
<td>Enzyme</td>
<td>Extrinsic</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor</td>
<td>Cofactor</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>IX</td>
<td>Plasma thromboplastin component; Christmas factor</td>
<td>Enzyme</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
<td>Enzyme</td>
<td>Common</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
<td>Enzyme</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
<td>Enzyme</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor</td>
<td>Enzyme</td>
<td>Common</td>
</tr>
</tbody>
</table>
Role of platelets in blood clotting

- Platelets (thrombocytes) have several functions in blood clotting:
  - Form platelet plug at the site of injury
  - Sites of activation of some clotting factors (II, X)
  - Provide the surface on which certain clotting factors bind (Va, Xa, II, Ca^{2+})
  - Sources of some clotting factors (XIII, PL)
Role of platelets in blood clotting

- Activated platelets release:
  - Fibrinogen
  - vWF
  - Factor V
  - Factor VIII
  - Platelet derived growth factor (PDGF) ~ promotes healing
  - Platelet factor IV – prevents formation of active thrombin inhibitor from heparin and anti-thrombin III.
  - ADP/ATP
  - Serotonin
  - Ca$^{2+}$
  - Dense core granules

α-granules

β-granules
The hormone *Thrombopoiten* (produced by liver) increases the rate of megakaryocytes in the bone marrow, stimulating them to produce more platelets.

Platelets deficiency can be due to many agents (drugs, some infections, ionizing radiation).

Individuals with thrombocytopenia (low platelets), bleed for a long time.
Function of Vitamin K

1. Formation of carboxyglutamate

- Vitamin K is essential for the functioning of several proteins involved in blood clotting (II, VII, IX and X)

- These proteins contain a unique modified glutamate residue, called carboxyglutamate (Gla).

- These proteins are synthesized as inactive precursors that are activated by the vitamin K-dependent carboxylase which converts glutamate in these proteins to carboxyglutamate forming mature clotting factors.
Function of Vitamin K (cont...)

1. Formation of carboxyglutaminate (cont...)

Dicumarol, Warfarin
Function of Vitamin K (cont...)

2. Interaction of prothrombin with platelets

- The Gla residue of prothrombin is a natural high affinity binder (chelator) of positively calcium ions, hence the designation of calcium as a co-factor (factor IV) in the schematic.

- The prothrombin-calcium complex is then able to bind to PLs essential for blood clotting on the surface of platelets.
Intrinsic system (surface contact)
- XII → XIIa
- XI → Xla

Extrinsic system (tissue damage)
- Tissue factor
- VIIa

Vitamin K dependant factors
- XIX
- VIII
- V

Fibrinogen
- II
- (Thrombin) → Fibrin

Coagulation cascade
Hemostasis is achieved through 4 coordinated processes:

- **Fibrin Deposition**
- **Platelet Plug**
- **Anti-Coagulant**
- **Fibrin Lysis**

Form the hemostatic plug → Fibrin Deposition → Platelet Plug → Anti-Coagulant → Fibrin Lysis → Limit and remove the hemostatic plug
Dissolution of fibrin clot: fibrinolysis
(The removal of fibrin from the blood)

- Clot is slowly dissolved by the “fibrin splitting” called **Plasmin**

- **Plasmin** gets trapped in clot and slowly dissolves it by breaking down the fibrin meshwork at various places, leading to the production of circulating fragments that are cleaved by other proteases or by the kidney and liver
Plasminogen is the inactive pre-cursor that is activated by activators in plasma:

1. Tissue plasminogen activator (t-PA)
2. Urokinase (to lesser extend)
   - Is produced as a precursor “prourokinase” by epithelial cells
   - Its main action is probably in the degradation of extracellular matrix
Dissolution of fibrin clot: fibrinolysis (cont...)

- Inactive t-PA is released from vascular endothelial cells following injury
- It binds to fibrin and is consequently activated
- Active t-PA converts plasminogen into plasmin

Dissolves the clot
## Inhibitors: ANTI-coagulants

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin III</td>
<td>• Most important (75%)&lt;br&gt;• Inhibits IXa, Xa, XIa and XIIa factors&lt;br&gt;• Is enhanced and accelerated by the presence of Heparin</td>
</tr>
<tr>
<td>Protein C</td>
<td>• Produced by liver; Vitamin K dependent&lt;br&gt;• Inhibits the cofactors VIIIa and Va&lt;br&gt;• Is enhanced by Protein S&lt;br&gt;• Needs to be activated by Thrombin (IIa)</td>
</tr>
<tr>
<td>Protein S</td>
<td>• Produced by liver; Vitamin K dependent&lt;br&gt;• Acts as a cofactor to Protein C to enhance its ability to degrade factors V and VIII</td>
</tr>
<tr>
<td>Heparin</td>
<td>• Acts both <em>in vivo</em> and <em>in vitro</em>&lt;br&gt;• Rapid onset few minutes&lt;br&gt;• Increases the rate of formation of irreversible complexes between antithrombin III and the serine protease clotting factors (i.e. inhibit the formation of thrombin)</td>
</tr>
<tr>
<td>Alpha 2-macroglobulins</td>
<td>• Contributes most of the remaining (25%) of antithrombin activity in plasma</td>
</tr>
</tbody>
</table>
# Artificial ANTI-coagulants

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin K antagonists</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Coumarin drug</strong></td>
<td>• Used only <em>in vivo</em></td>
</tr>
<tr>
<td></td>
<td>• Inhibit carboxylation of Glu residues in prothrombine and factors VII, IX, X</td>
</tr>
<tr>
<td><strong>Dicumarol</strong></td>
<td>• Used only <em>in vivo</em> as anticoagulant to prevent thrombosis in patients with a tendency to form blood clot</td>
</tr>
<tr>
<td></td>
<td>• Slow onset of action 2-3 days but long duration 4-6 days</td>
</tr>
<tr>
<td><strong>Citrate oxalate</strong></td>
<td>• Used only <em>in vivo</em></td>
</tr>
<tr>
<td></td>
<td>• Removes Ca$^{2+}$</td>
</tr>
<tr>
<td><strong>Defibrination of blood</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Break down of fibrin threads once formed by continuous shaking or by glass rod</td>
</tr>
<tr>
<td><strong>Heparin</strong></td>
<td></td>
</tr>
</tbody>
</table>
## Coagulation factor disorders

<table>
<thead>
<tr>
<th>Inherited bleeding disorders</th>
<th>Acquired bleeding disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>◦ Hemophilia A and B</td>
<td>◦ Liver disease</td>
</tr>
<tr>
<td>◦ Von Willebrand disease</td>
<td>◦ Vitamin K deficiency</td>
</tr>
<tr>
<td>◦ Other factor deficiencies</td>
<td></td>
</tr>
</tbody>
</table>
Coagulation factor disorders (cont...)

1- Hemophilia A and B

Are the best-known coagulation factor disorders

<table>
<thead>
<tr>
<th></th>
<th>Hemophilia A</th>
<th>Hemophilia B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation factor deficiency</td>
<td>Factor VIII</td>
<td>Factor IX</td>
</tr>
<tr>
<td>Inheritance</td>
<td>X-linked recessive</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td>Incidence</td>
<td>1/10,000 males</td>
<td>1/50,000 males</td>
</tr>
</tbody>
</table>
Coagulation factor disorders (cont...)

2- von Willebrand Disease

- It is the most common hereditary bleeding disorder and is characterized as being inherited autosomal recessive or dominant.

- In this disease there is a defect in von Willebrand factor (vWF) which:
  1. acts as a carrier for factor VIII
  2. mediates the binding of glycoprotein Ib (GPIb) to collagen
Coagulation factor disorders (cont...)

2- von Willebrand Disease

- This binding helps the activation of platelets and formation of primary hemostasis

- vWD is characterized by excessive bleeding in infants because platelets fail to form hemostatic plug
### Coagulation factor disorders (cont...)

## 3- Deficiency of Vitamin K

<table>
<thead>
<tr>
<th>Source of vitamin K</th>
<th>Green vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesized by intestinal flora</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Required for synthesis</th>
<th>Factors II, VII, IX ,X</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>contribute to bleeding disorders</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Causes of deficiency</th>
<th>Malnutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biliary obstruction</td>
</tr>
<tr>
<td></td>
<td>Malabsorption</td>
</tr>
<tr>
<td></td>
<td>Antibiotic therapy</td>
</tr>
</tbody>
</table>
Leukocytes (WBC’s)
Two major components of blood: liquid phase and formed elements

- **Plasma**
  - Fluid portion of blood
  - Contains:
    - 91 - 92% of water
    - Albumin & globulin
    - Crucial hormones & clotting factors

- **Red Cells**
  - Transports oxygen from the lungs to all tissues of the body and return carbon dioxide back to the lungs

- **White Cells**
  - Protect against diseases & infections

- **Platelets**
  - Small plate-shaped cells that cluster together to help form blood clots when bleeding occurs
All new WBCs except for lymphocytes are produced in the bone marrow (that also give rise to erythrocytes and platelets). Most new lymphocytes are produced by colonies of cells in lymphoid tissues, such as lymph nodes.
Leukocytes (WBC’s)

Mobile units of body’s defense system:

- **“Seek and Destroy” Functions:**
  - Destroy invading microorganisms
  - Destroy abnormal cells (i.e., cancer)

- **Clean up cellular debris (phagocytosis):**
  - Assist in injury repair

- Each WBC has a specific function
Leukocytes (WBC’s) (Cont...)

<table>
<thead>
<tr>
<th>Leukocyte group</th>
<th>Subgroup</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>granulocytes</td>
<td>neutrophils</td>
<td>destroy small organisms</td>
</tr>
<tr>
<td></td>
<td>basophils</td>
<td>secrete histamine, mediating inflammatory response, and platelet activating factor</td>
</tr>
<tr>
<td></td>
<td>eosinophils</td>
<td>destroy parasites allergic reaction</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>B lymphocytes</td>
<td>synthesize antibody</td>
</tr>
<tr>
<td></td>
<td>T lymphocytes</td>
<td>participate in the specific immune response</td>
</tr>
<tr>
<td>monocytes</td>
<td>macrophages</td>
<td>destroy invading organisms</td>
</tr>
</tbody>
</table>
Leukocytes (WBC’s) (Cont…)

- Five Types

- Classified according to the presence or absence of granules and the staining characteristics of their cytoplasm.

- Leukocytes appear brightly colored in stained preparations, they have a nuclei and are generally larger in size than RBC’s.
Types of WBC’s

Are classified in 3 main classes

Agranulocytes

- Lymphocyte
- Monocyte

Granulocytes

- Eosinophil
- Basophil
- Neutrophil
Granulocytes (Polymorphonuclear leukocytes): have 2 types of granules in their cytoplasm: the specific granules (specific functions) and azurophilic granules (lysosomes)

- Neutrophils
- Eosinophils
- Basophils
Agranulocytes: do not have specific granules, but they do contain azurophilic granules in their cytoplasm

- Lymphocytes
- Monocytes
1. Neutrophils (cond...)

- Constitute 60-70% of circulating WBC’s
- Have an average diameter of 12-15 µm
- Several lobes in nucleus (2-5 segments) linked by fine threads chromatin
- Also contain glycogen (source of energy)
- Stain light purple with neutral dyes
1. Neutrophils (cont...)

- Granules are small and numerous
- Highly mobile/very active
- **Diapedesis:** Can leave blood vessels and enter tissue space
- Short lived cells: life span of 6-7h in blood and 1-4 days in connective tissues
- **Function:** Phagocytosis (contain several lysosomes) and play a major role of acute inflammation
2. Eosinophils

- 2-4% in normal blood
- Large, numerous granules
- Typical bilobed nuclei
- Are about 12-17 \( \mu m \) in size, pale blue colour
- Found in lining of respiratory and digestive tracts
2. Eosinophils (cont...) 

- Persist in the circulation for 8–12 hours

**Functions:**

- Important functions involve protections against infections caused by parasitic worms and involvement in allergic reactions
- Secrete anti-inflammatory substances in allergic reactions
3. Basophils

- Least numerous, less than 1% of blood WBC’s
- They are about 12-15 µm diameter
- They contain many large, rounded, dark purplish black granules
- Their nucleus is divided into irregular lobes
3. Basophils (cont...)

- Diapedesis
- Contain histamine and heparin (inflammatory chemical)
- **Function:** Like eosinophils, basophils play a role in both parasitic infections and allergies
1. Lymphocytes

- Constitute 28% of WBC’s
- Small lymphocytes (6-8 μm); medium-sized lymphocytes (small number) and large lymphocytes (18 μm)
- Large nuclei/small amount of cytoplasm
- Color pale-blue
1. Lymphocytes (cont...)

- Only type of WBC’s that return from the tissue back to blood after diapedesis
- Vary in life span: some live only a few days (~3 days), others survive in circulating blood for many years (4-5 years)
1. Lymphocytes (cont...)

- **Function**: immune responses and memory, mainly found in lymph tissue

- Two types:
  - **T lymphocytes** attack an infect or cancerous cell
  - **B lymphocytes** produce antibodies against specific antigens (foreign body)
2. Monocytes

- Largest of WBCs (12-20µm)
- Dark kidney bean shaped nuclei
- Cytoplasm is basophilic and frequently contain very fine azurophilic granules
- In tissues differentiate into macrophages
2. Monocytes (cont...)

**Function:** phagocytosis

- evident in chronic infections – Tuberculosis
- defense vs. viruses and certain bacteria
- activate lymphocytes
WBC Numbers

- Doctors look at WBC numbers.
- Clinics will count the number of WBC’s in a blood sample, this is called differential count.
- A decrease in the number of white blood cells is leukopenia.
- An increase in the number of white blood cells is leukocytosis.
Metabolism of leukocytes

- They have aerobic glycolysis and active pentose phosphate pathway (NADPH)

- During phagocytosis of bacteria, there is an increase of $O_2$ consumption (respiratory burst: the rapid release of reactive oxygen species) and superoxide radical $O_2^-$ (involved in killing the bacteria) is formed.
Phagocytic leukocytes use NADPH as a substrate for the NADPH-oxidase enzyme, which contributes to the killing of ingested microorganisms.

\[
\text{NADPH} + \text{A} + \text{O}_2 \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + \text{AH} + \text{O}_2^- \\
2\text{H}^+ + 2\text{O}_2^- \xrightarrow{\text{Acidic pH SOD}} 2\text{H}_2\text{O}_2 + \text{AH} + \text{O}_2^-
\]

Helps to kill microorganisms
Metabolism of leukocytes (cont...)

- Active leukocytes release $O_2^-$ ions and $H_2O_2$ to surrounding tissues in areas of inflammations.

- Superoxide dismutase, catalase and glutathione peroxidase are normal antioxidant enzymes that help to protect the body against the toxic effect of $O_2$ ions and $H_2O_2$. 
Immunoglobulins ($\gamma$ globulins)
The body defends itself from infections and other foreign substances in a number of ways.
The Immune System is the Third Line of Defense Against Infection

<table>
<thead>
<tr>
<th>Nonspecific Defense Mechanisms</th>
<th>Specific Defense Mechanisms (Immune System)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First line of defense</strong></td>
<td><strong>Third line of defense</strong></td>
</tr>
<tr>
<td>Skin</td>
<td>Lymphocytes (cellular immunity)</td>
</tr>
<tr>
<td>Mucous membranes</td>
<td>Antibodies (Humoral immunity)</td>
</tr>
<tr>
<td>Secretions of skin and mucous membranes</td>
<td>· Phagocytic white blood cells</td>
</tr>
<tr>
<td></td>
<td>· Antimicrobial proteins</td>
</tr>
<tr>
<td></td>
<td>· The inflammatory response</td>
</tr>
</tbody>
</table>

**Mechanical barriers:**

In contact with external substances or organisms

Fast but not enough
**Immunoglobulins**

- **Definition:** Glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies
- **Produced by:** B-lymphocytes in response to exposure to antigen
- **React specifically with antigen**
- **Five classes of Antibodies:** IgG; IgM; IgA; IgD; IgE
Antibody structure

- All Iggs have a similar basic structure
- Glycoproteins made up of 4 polypeptide chains (IgG):
  1. Two light (L) polypeptide chains (25 kDa)
  2. Two heavy (H) polypeptide chains (50 kDa)
- The four chains are linked by disulfide bonds
Antibody structure

- Terminal portion of L-chain contains part of antigen binding site
- Terminal portion of H-chain participate in antigen binding site
Antibody structure

- An antibody molecule is composed of two identical Ig heavy chains (H) and two identical light chains (L), each with a variable region (V) & constant region (C).

- The Variable regions of the heavy chains = $V_H$
- The Variable region of the light chains = $V_L$
- Constant region of the light chain = $C_L$
- Constant regions of the heavy chain = $C_H$
Variable (V) Region

- **V-region** lies in terminal portion of molecule
- **V-region** shows wide variation in amino acid sequences
- **Hypervariable** region form region complementary to Ag determinant
- It is responsible for antigen binding
Constant (C) Region

- **C-region** lies in carboxyl or terminal portion of molecule
- **C-region** shows an unvarying amino acid sequence
- It is responsible for biologic functions
Hinge region

- This is the region at which the arms of the antibody molecule forms a Y.
- It is called the **hinge region** because there is some flexibility in the molecule at this point.
Greek letters are used to name the heavy and light chain constant regions

\( \gamma, \mu, \alpha, \delta, \varepsilon \) for the heavy chains

\( \kappa, \lambda \) for the light chains

The heavy chain determines the class of the Ig, thus

- If the heavy chain is \( \gamma \) the class is \( \text{IgG} \)
- If the heavy chain is \( \mu \) the class is \( \text{IgM} \)
- If the heavy chain is \( \alpha \) the class is \( \text{IgA} \)
- If the heavy chain is \( \delta \) the class is \( \text{IgD} \)
- If the heavy chain is \( \varepsilon \) the class is \( \text{IgE} \)

Different heavy chains provide different functions and distribution; there is no known difference in function for \( \kappa \) and \( \lambda \)
1. IgG

- **Percentage serum antibodies:** 80% (Major serum Ig)
- **Major Ig in extravascular spaces**
- **Location:** Blood, lymph, intestine
- **Half-life in serum:** 23 days
- **Placental Transfer:** The only placental transfer Ig
- **Known Functions:** Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn.
Human Immunoglobulin Classes

2. IgM

- **Percentage serum antibodies:** 5-10%
- First Ig made by fetus and B cells
- Present in colostrum and mother milk protect newly born.
- **Location:** Blood, lymph, B cell surface
- **Half-life in serum:** 5 days
- **Placental Transfer:** No
- **Known Functions:**
  - First antibodies produced during an infection (the major Igs during primary immune response)
  - Effective against microbes and agglutinating antigens.
Human Immunoglobulin Classes

3. IgA

- Percentage serum antibodies: 10-15%
- Location: Secretions (tears, saliva, intestine, milk), blood and lymph
- Half-life in serum: 6 days
- Placental Transfer: No

Known Functions:
- Localized protection of mucosal surfaces.
- Provides immunity to infant digestive tract.
4. IgD

- **Percentage serum antibodies:** 0.2%
- **Location:** B-cell surface, blood, and lymph
- **Half-life in serum:** 3 days
- **Placental Transfer:** No

**Known Functions:**
- In serum function is unknown
- On B cell surface, initiate immune response
5. IgE

- **Percentage serum antibodies**: 0.002%
- **Location**: Bound to mast cells and basophils throughout body Blood.
- **Half-life in serum**: 2 days
- **Placental Transfer**: No
- **Known Functions**:
  - Associated with allergic reactions.
  - Possibly lysis of worms.
## Primary and Secondary antibody response

<table>
<thead>
<tr>
<th>Primary antibody response</th>
<th>Secondary antibody response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First exposure</strong> to antigen</td>
<td><strong>Subsequent exposure</strong></td>
</tr>
<tr>
<td>lag period: \textbf{days or weeks} (slow onset)</td>
<td>Lag period: \textbf{hours} (rapid onset)</td>
</tr>
<tr>
<td>Small amount Ig: low Ab level with gradual increase</td>
<td>\textbf{large amount Ig:} high Ab level with rapid increase</td>
</tr>
<tr>
<td>Ab \textbf{Persist for short duration} (Weeks then decline rapidly)</td>
<td><strong>Persist for long periods</strong> (months or years)</td>
</tr>
<tr>
<td>Antibody is \textbf{IgM}</td>
<td>Antibody is \textbf{IgG}</td>
</tr>
</tbody>
</table>
Primary and Secondary antibody response

- Primary response to Ag
- Secondary response to Ag

Amount of antibodies in serum vs. Time (months)

1st injection of Ag
2nd injection of Ag