HEMATOLOGY
&LYMPH SYSTEM

Histology Handout

Number	1
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Blood, Lymphatic, and Immune Systems

Introduction

Homeostasis, or a "steady state," is a continual balancing act of the body systems to provide an internal environment that is comparable with life. The two liquid tissues of the body, the **blood** and l**ymph** have separate but interrelated functions in maintaining this balance. They combine with a third system, the **immune**, to protect the body against **pathogens** that could threaten the organism's viability. The **blood** is responsible for the following:

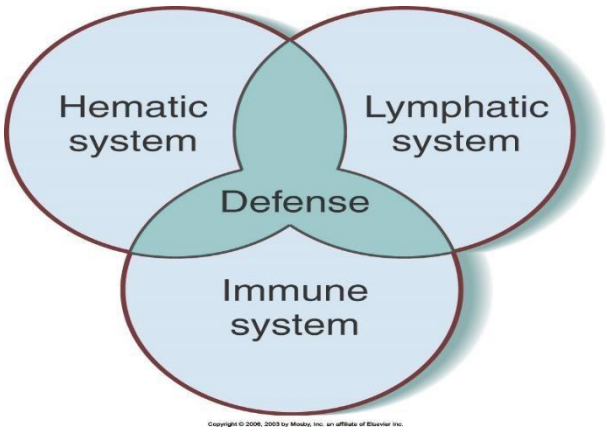
- Transportation of gases (oxygen O2) and carbon dioxide (CO2), chemical substances (hormones, nutrients, salts), and cells that defend the body.
- Regulation of the body's fluid and electrolyte balance, acid-base balance, and body temperature.
- Protection of the body from infection.
- Protection of the body from loss of blood by the action of clotting.

The **lymph system** is responsible for the following:

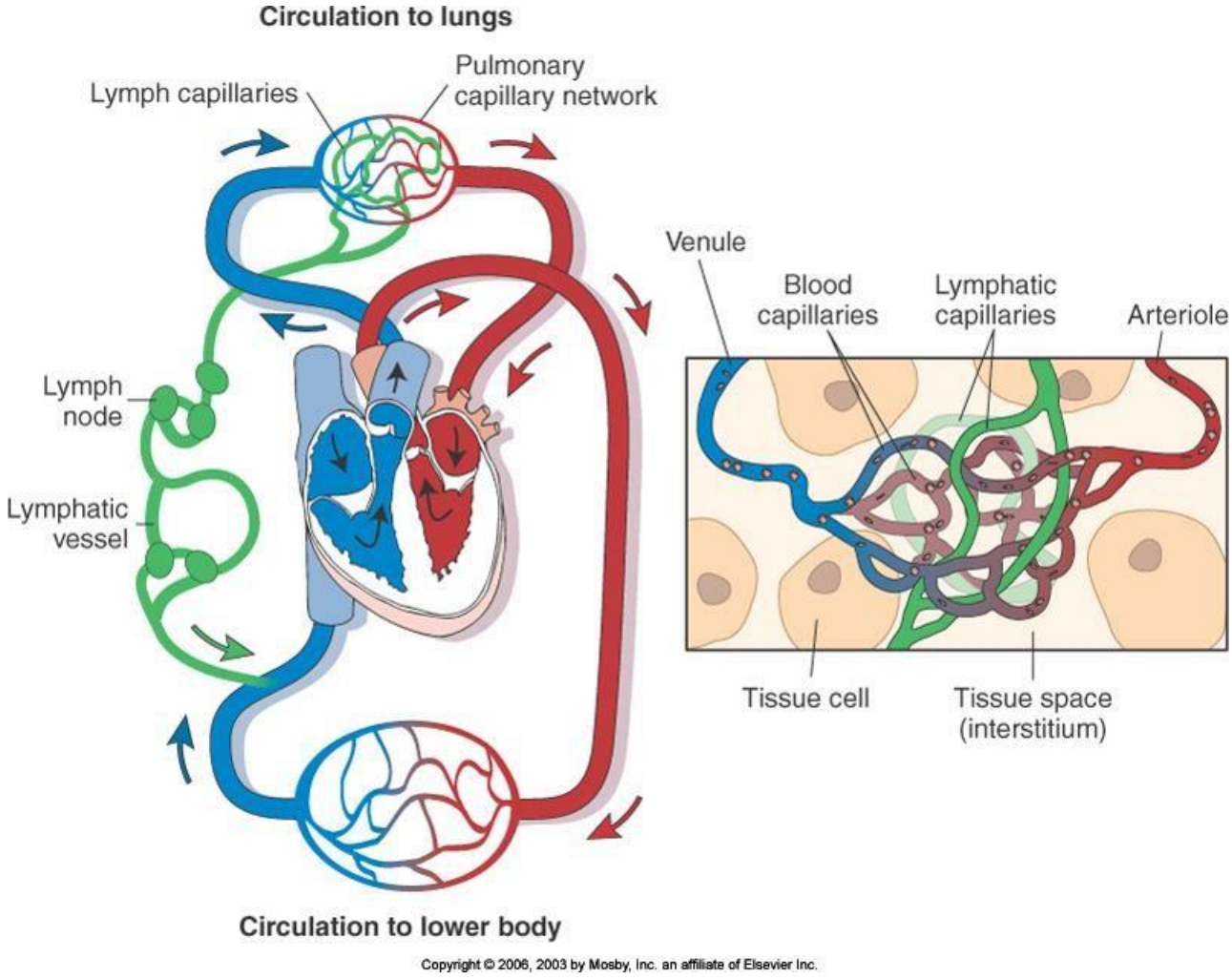
- Cleansing the cellular environment
- Returning proteins and tissue fluids to the blood (drainage)
- Providing a pathway for the absorption of fats and fat-soluble vitamins into the bloodstream.
- Defending the body against disease.

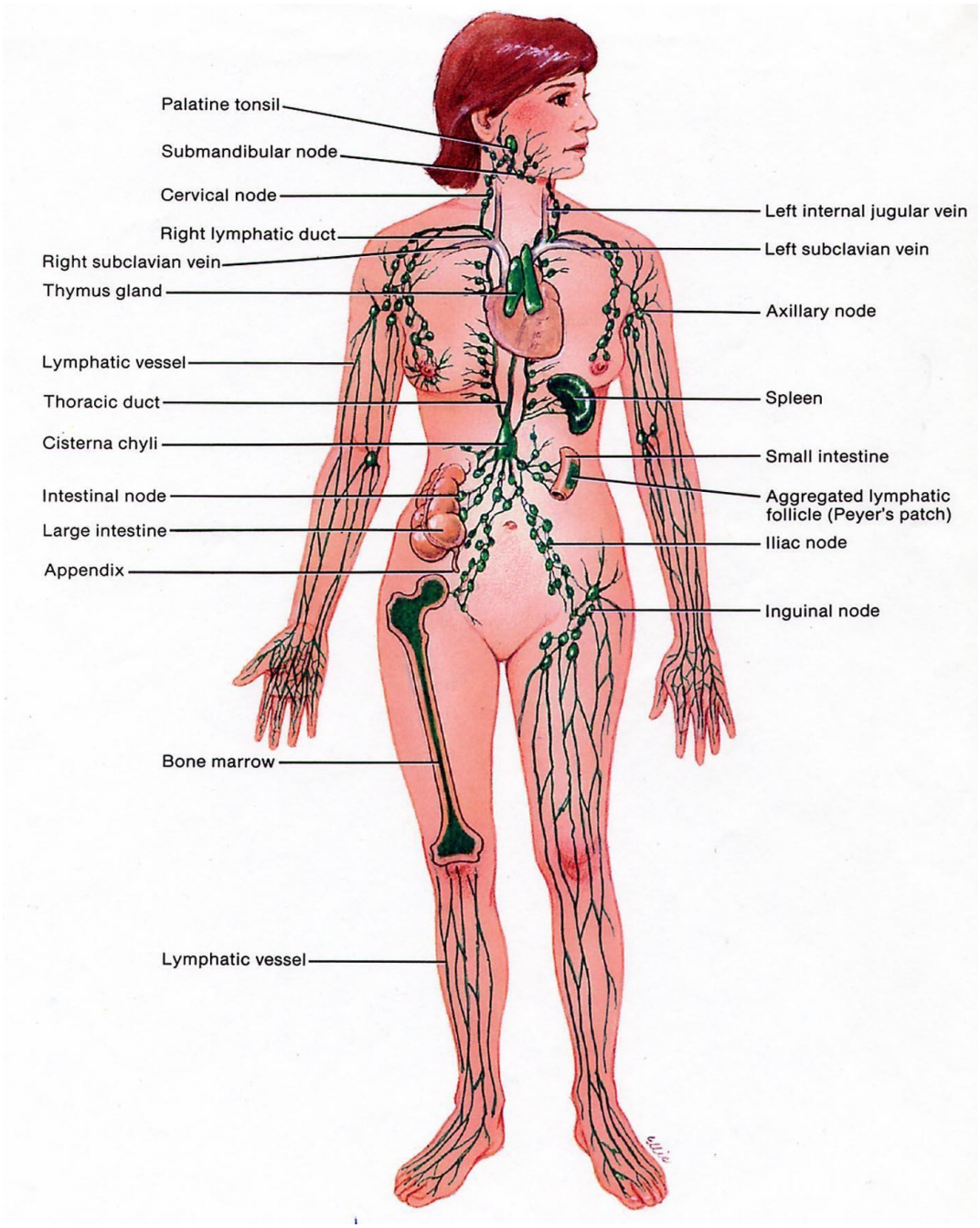
The **immune system** is responsible for the following:

- Defending the body against disease via the immune response



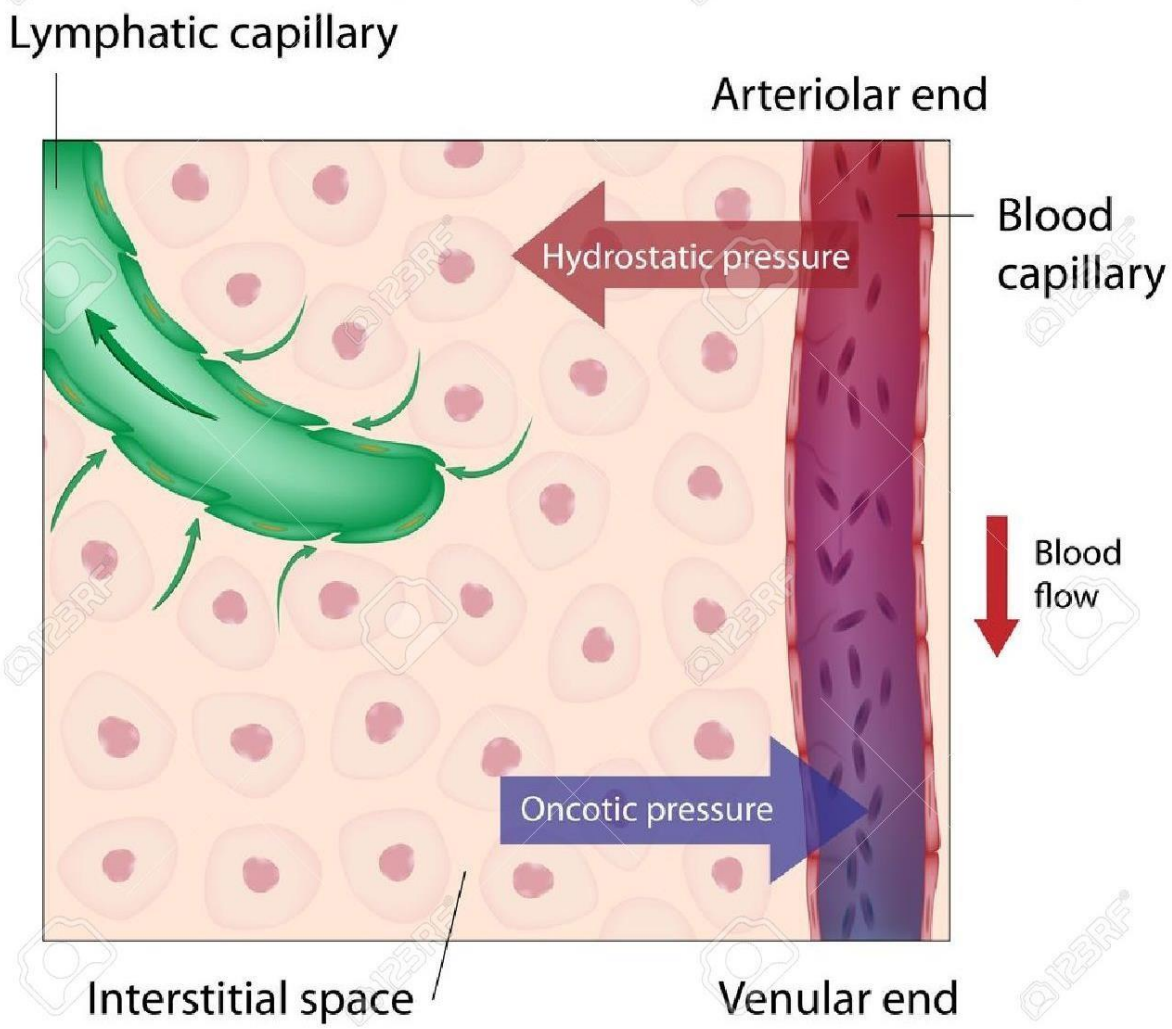
The hematic and lymphatic systems flow through separate yet interconnected and interdependent channels. Both are systems composed of vessels and the liquids that flow through them. The **immune system**, a very complex set of levels of protection for the body, includes blood and lymph cells.





The above graphics show the relationship of the lymphatic vessels to the circulatory system. Note the close relationship between the distribution of the lymphatic vessels and the venous blood vessels.

Filtration in capillaries creates tissue fluid, most of which returns almost immediately to the blood in the capillaries by osmosis. Some tissue fluid, however, remains in interstitial spaces and must be returned to the blood by way of the lymphatic vessels. Without this return, blood volume and blood pressure would very soon decrease. Tissue fluid is drained by the lymphatic capillaries and transported by a series of larger lymphatic vessels into the circulation.


Lymphatic capillary
Arteriolar end
Blood capillary
Hydrostatic pressure
Blood flow
Oncotic pressure
Interstitial space
Venular end

Hematic System (Blood)

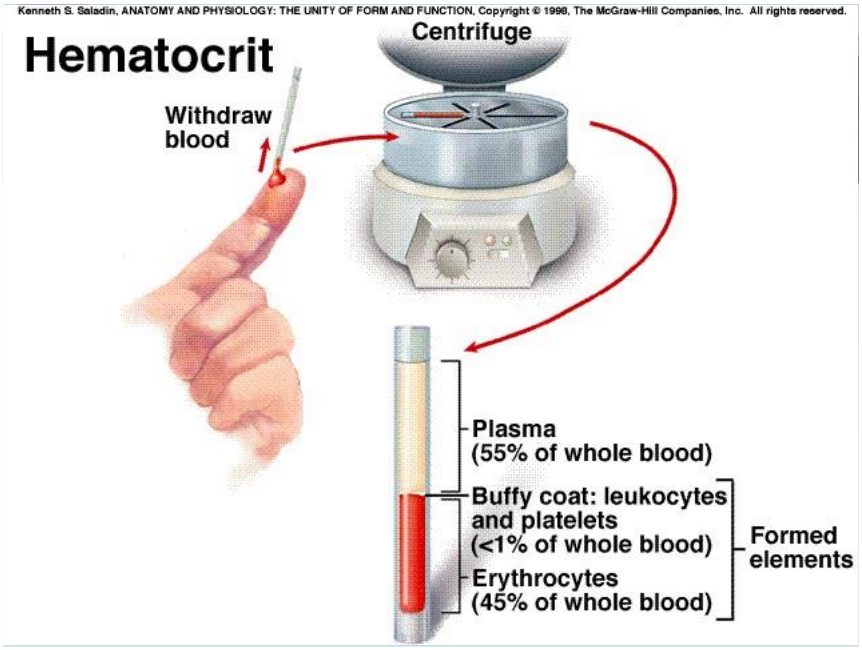
What is Blood?

Blood is the fluid that circulates in the peripheral vascular system of humans. Blood is a tissue. It is classified as a special type of connective tissue. Blood is considered a connective tissue for two basic reasons: (1) embryologically, it has the same origin (mesoderm) as do the other connective tissue types and (2) blood connects the body systems together bringing the needed oxygen, nutrients, hormones and other signaling molecules, and removing wastes.

Characteristics of blood

Blood has distinctive physical characteristics:

Amount: an adult person has 4 to 6 liters of blood, depending on his or her size (around 7-8% of weight). Of the total blood volume in the human body, 38% to 48% is composed of the various blood cells, also called "formed elements". The remaining 52% to 62% of the blood volume is plasma, the liquid portion of blood.



Color: blood color varies; arterial blood is bright red because it contains high levels of oxygen. Venous blood have given up much of its oxygen in tissues, and has a darker, dull red color. This may be important in the assessment of the source of bleeding. If blood is bright red, it is probably from a severed artery, and the dark red blood is probably venous blood.

pH: the normal pH range of blood is 7.35 to 7.45, which is slightly alkaline. Venous blood normally has a lower pH than does arterial blood because of the presence of more carbon dioxide.

Viscosity: this means thickness or resistance to flow. Blood is about three to five times thicker than water. Viscosity is increased by the presence of blood cells and the plasma proteins, and this thickness contributes to normal blood pressure.

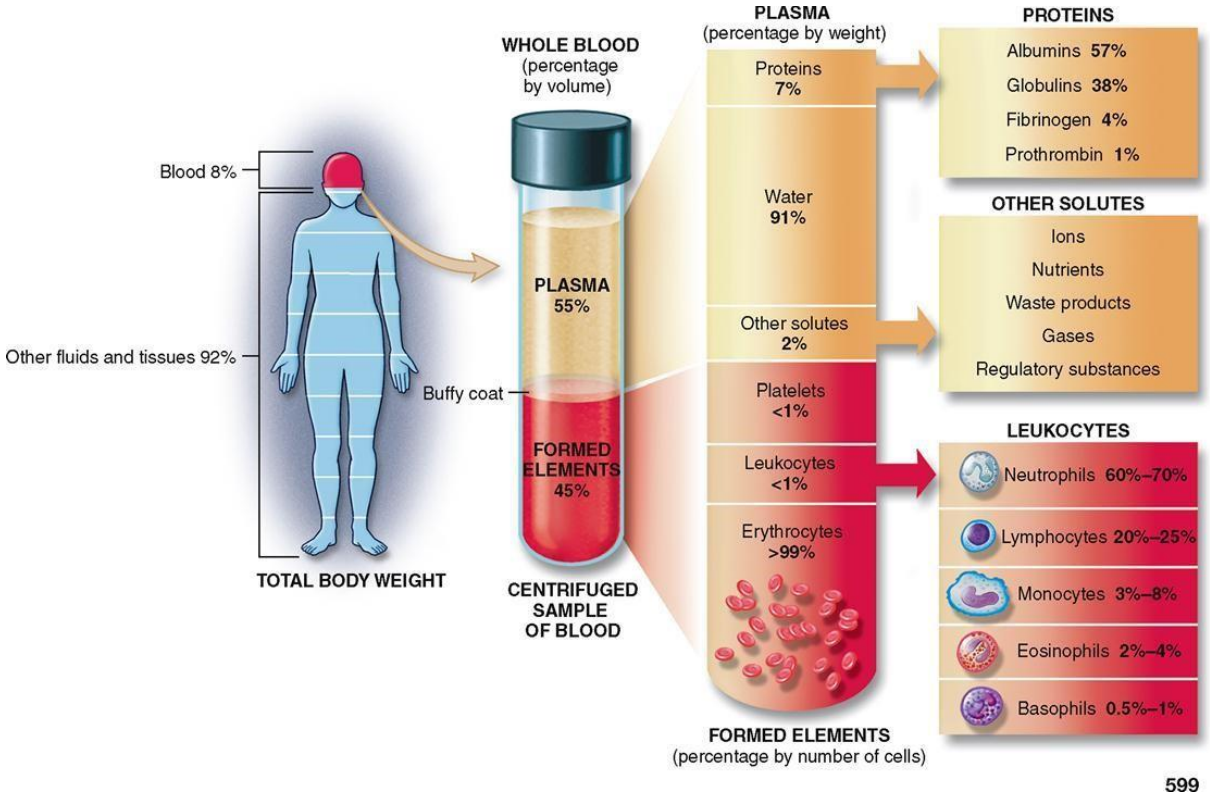
Plasma

Plasma is the liquid part of blood and is approximately 90% water. The solvent ability of water enables the plasma to transport many types of substances (water is often regarded as a universal solvent). Nutrients absorbed in the digestive tract are circulated to all body tissues, and waste products of the tissues circulate through the kidneys and are excreted in urine. Hormones produced by endocrine glands are carried in the plasma to their target organs, and antibodies are also transported in plasma.

Most of the carbon dioxide produced by cells is carried in the plasma in the form of bicarbonate ions (HCO_3^-). When the blood reaches the lungs, the CO_2 is re-formed, diffuses into alveoli and is exhaled.
Plasma also carries blood heat. Blood is warmed by flowing through active organs such as the liver and muscles. This heat is distributed to cooler parts of the body as blood continues to circulate.

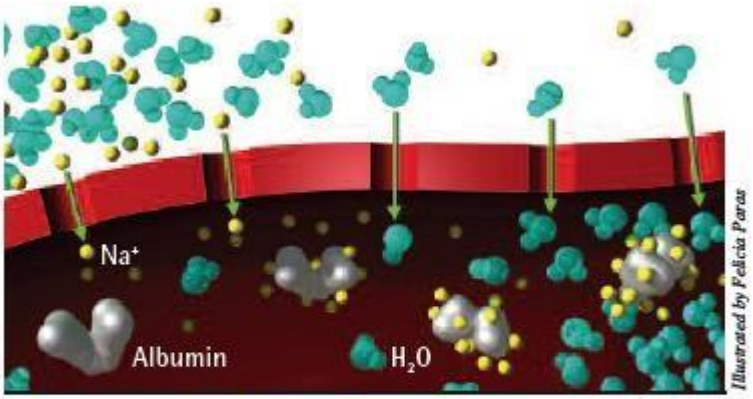
Plasma is composed of the following:

1. Water, or H2O (90%)
2. Inorganic substances (calcium, potassium, sodium)
3. Organic substances (glucose, amino acids, fats, cholesterol, hormones)
4. Waste products (urea, uric acid, ammonia, creatinine)
5. Plasma proteins (serum albumin, serum globulin, and two clotting proteins: fibrinogen and prothrombin)



Plasma proteins

Albumin is the most abundant plasma protein. It is synthesized by the liver. Albumin contributes to the colloid osmotic pressure of blood, which pulls tissue fluid into capillaries. This is important to maintain normal blood volume and blood pressure. Albumin also serves as a transport protein, particularly for fatty acids and several hydrophobic steroid hormones.



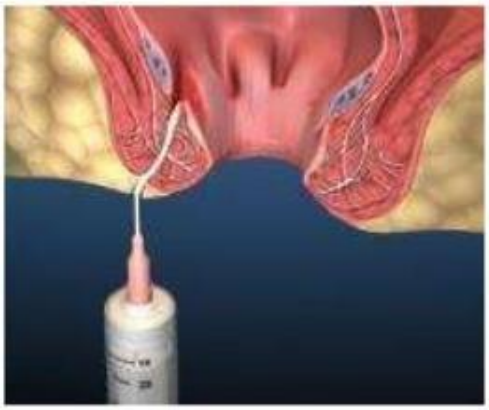
▲ Albumin does not readily move through normal capillary pores, while water and smaller biologic structures move freely. Sodium is highly attracted to albumin, and together, they help maintain COP by attracting water into the intravascular space.

Other plasma proteins are called **globulins**. Alpha and beta globulins are synthesized by the liver and act as carriers for molecules such as fats. The gamma globulins are antibodies produced by lymphocytes. Antibodies initiate the destruction of pathogens and provide us with immunity.

The clotting factors **prothrombin**, **fibrinogen**, and others are also synthesized by the liver and circulate until activated to form a clot in a ruptured or damaged blood vessel. Fibrinogen is soluble in plasma. It is converted into fibrin as part of the clotting cascade. Fibrin fibers form the frame for the blood clot.

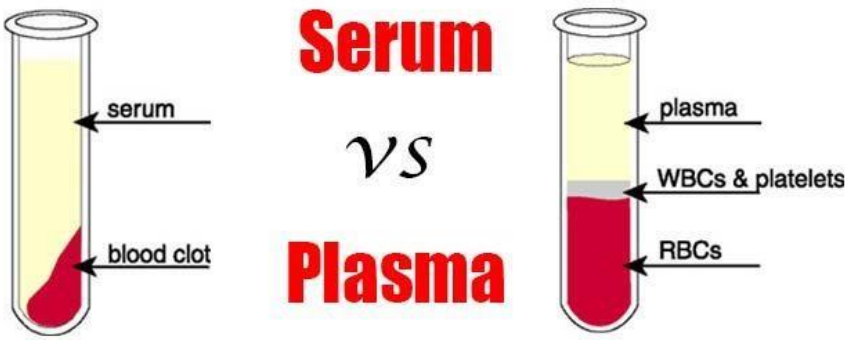
Fibrin Sealant

Fibrin sealant is a form of surgical glue. The fibrin sealants are comprised of purified, virus-inactivated human fibrinogen, human thrombin, and sometimes added components, such as virus-inactivated human factor XIII and bovine aprotinin. Fibrin sealants are the most effective tissue adhesives currently available, and they are biocompatible and biodegradable. The drawing below shows the use of fibrin sealant in the treatment of anal fistula (Fistula-in-ano).



Serum

Serum is plasma minus the clotting proteins. Serology is the branch of laboratory medicine that studies blood serum for evidence of infection by evaluating antigen-antibody reactions in vitro.

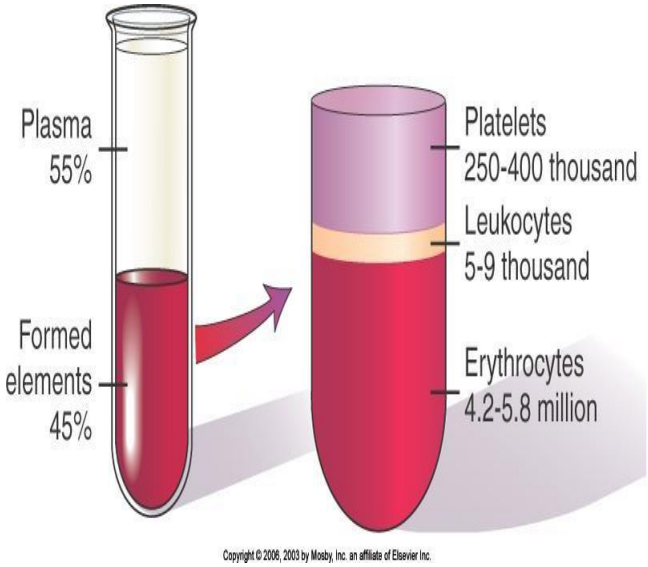


Serum = Plasma – Clotting Factors

<u>Blood Cells (Formed elements)</u>

The solid portion of blood is composed of three different types of cells:

- **Erythrocytes** - also called red blood cells (RBCs).
- **Leukocytes** - also called white blood cells (WBCs).
    - **Granulocytes / Polymorphonuclear leukocytes** (Neutrophils, Eosinophils, Basophils)
    - **Mononuclear leukocytes / Agranulocytes** (Lymphocytes, Monocytes)
- **Thrombocytes** - also called **platelets**.



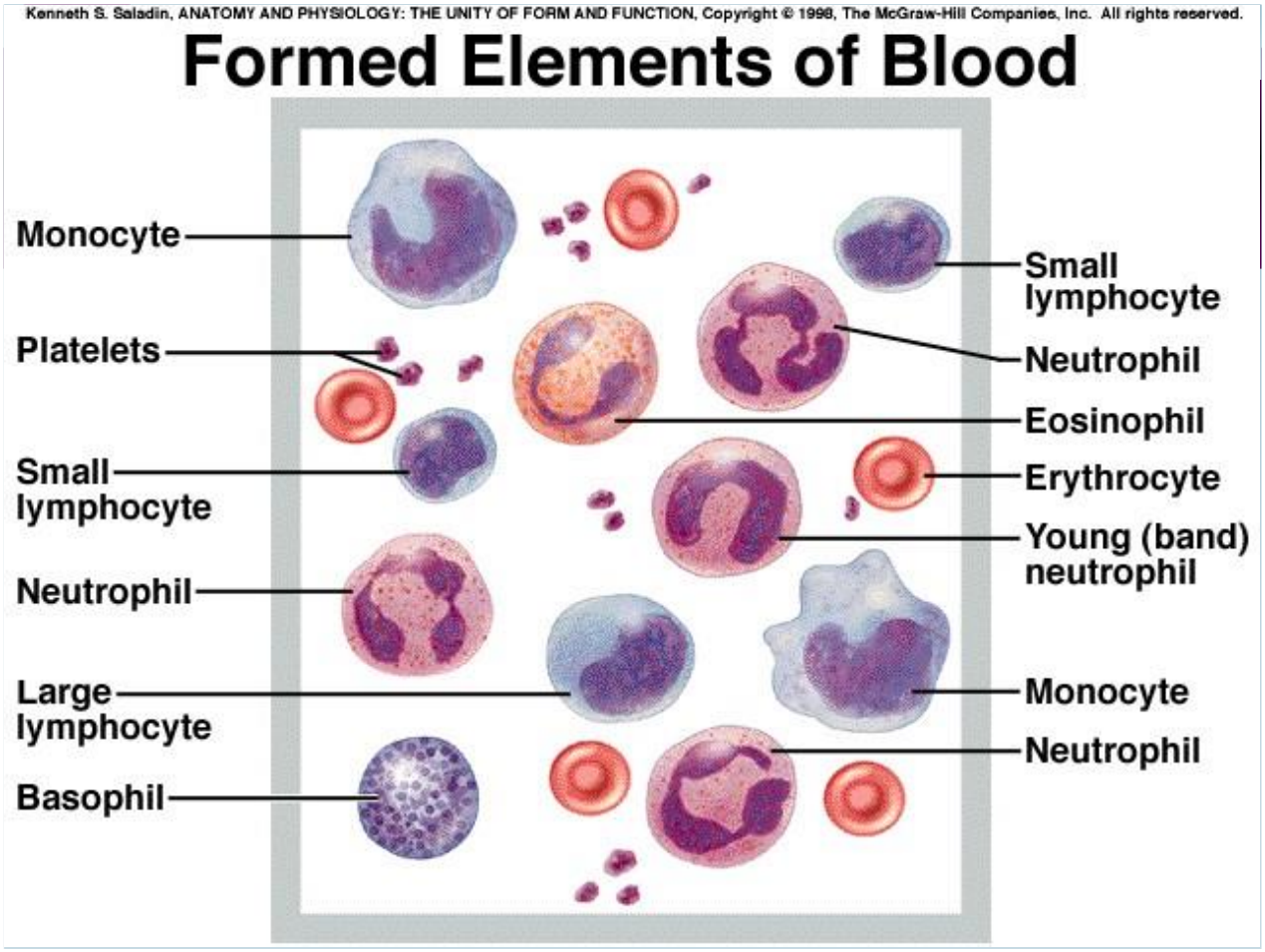
Peripheral Blood Film (Blood Smear)

The peripheral blood film (PBF) is a laboratory work-up that involves cytology of peripheral blood cells smeared on a slide. As basic as it is, PBF is invaluable in the characterization of various clinical diseases. Despite advances in hematology automation and application of molecular techniques, the PBF has remained a very important diagnostic test to the hematologist. A good quality smear, thorough examination and proper interpretation in line with patient's clinical state should be ensured by the hemato-pathologist.

Blood film procedure (in the Lab)

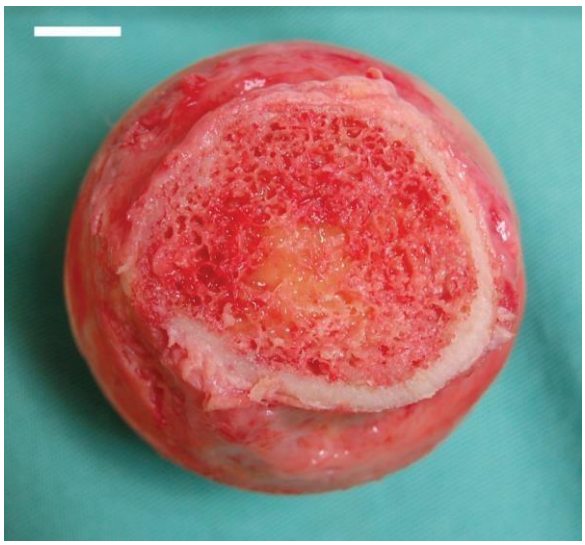
A drop of well mixed blood (minimum of 10 gentle inversions) is placed on the base of a slide close to one end (about 1 cm from the edge) with a pipette/capillary tube. A spreader slide with chipped edges is placed on the base slide in front of the blood and moved backwards to touch the drop of blood which makes the blood spread along the base slide-width. The spreader slide should have a smooth end to prevent the tail end of the smear from being irregular. Then, a smear is made with the spreader inclined at an angle of about 30 to 45 degrees to the blood. The smear is properly air dried. Then proceed to label the slide with pencil or crayon on the frosted end of the slide. The dried smear is fixed with absolute methanol or ethyl alcohol and stained with a

Rowmanosky stain (polychromatic stain). A properly air dried smear should be fixed within 4 hours of preparation but preferably within one hour. Good fixation requires about 10 to 20 minutes. Romanosky stains are mixtures of acidic dye and basic dyes that give a differential staining of the different cellular components. A commonly used stain is Leishman stain which is composed of polychrome methylene blue (basic component) and eosin (acidic component), in a methanolic mixture (fixation). May-Grunwald Giemsa, Jenner, or WrightGiemsa stain can also be used. The intensity of the staining varies with the duration of stain contact time and concentration of the stain. The smear is floored with stain for about 5-10 minutes, then double diluted with buffered water and allowed for another 5–10 minutes for the cells to pick the stain. After this, the slide is properly rinsed under running water.



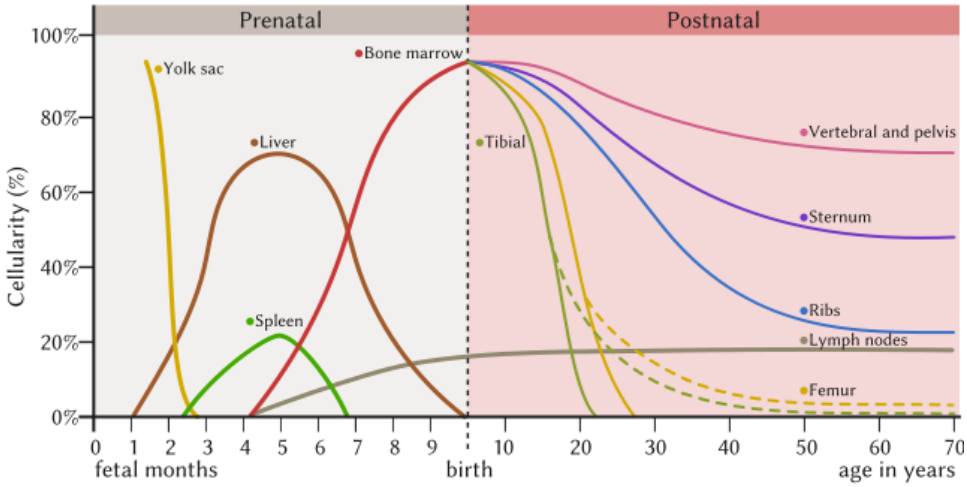
Hematopoeisis

Hematopoiesis or hemopoiesis is the production of blood cellular components. Blood cells are produced in **hemopoietic tissues**, of which there are two: **red bone marrow**, found in flat and irregular bones, and **lymphatic tissue**, found in the spleen, lymph nodes, and thymus gland.



Red bone marrow fills the head of the femur, and a spot of yellow bone marrow is visible in the center. The white reference bar is 1 cm.

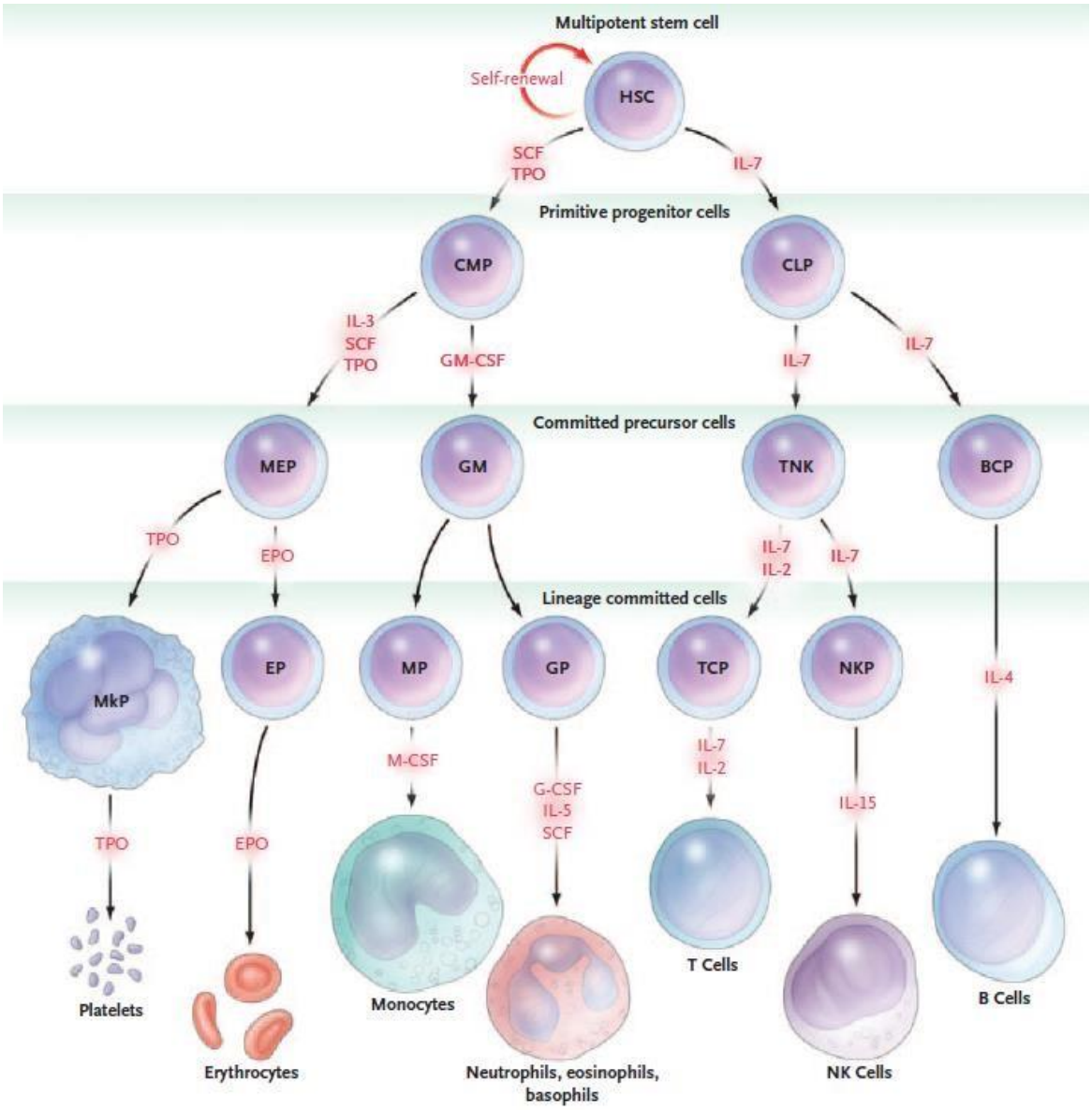
In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation of lymphoid cells occurs in the spleen, thymus, and lymph nodes.
In children, hematopoiesis occurs in the marrow of the long bones such as the femur and tibia.
In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum (flat bones).

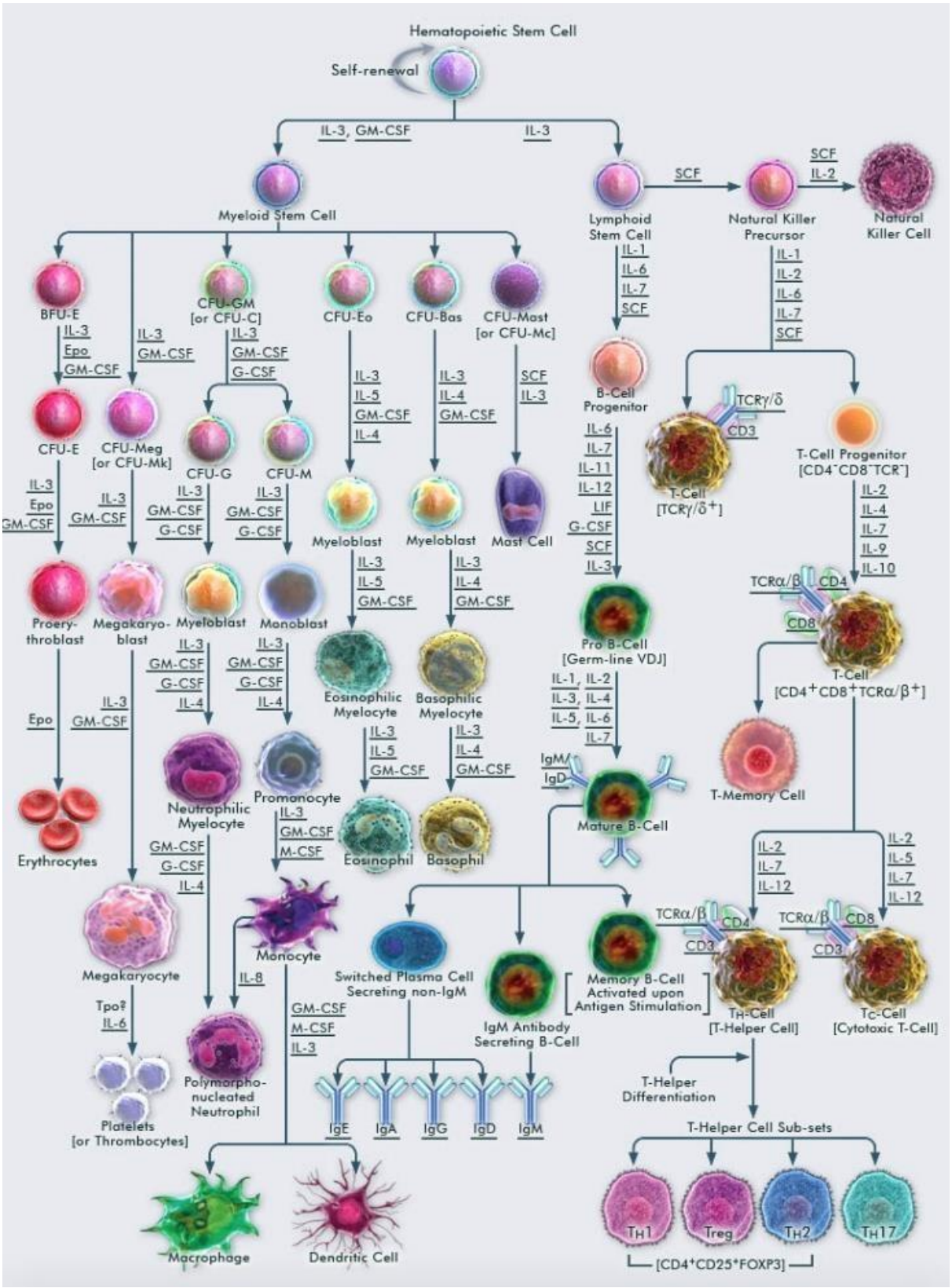


In some cases, the liver, thymus, and spleen may resume their hematopoietic function, if necessary. This is called **extramedullary hematopoiesis**. It may cause these organs to increase in size substantially. During fetal development, since bones and thus the bone marrow develop later, the liver functions as the main hematopoetic organ. Therefore, the liver is enlarged during development.

Blood-cell development progresses from a hematopoietic stem cell (HSC), which can undergo either self-renewal or differentiation into a multilineage committed progenitor cell: a common lymphoid progenitor (CLP) or a common myeloid progenitor (CMP).
These cells then give rise to more-differentiated progenitors, comprising those committed to two lineages that include T cells and natural killer cells (TNKs), granulocytes and macrophages (GMs), and megakaryocytes and erythroid cells (MEPs).
Ultimately, these cells give rise to unilineage committed progenitors for B cells (BCPs), NK cells (NKPs), T cells (TCPs), granulocytes (GPs), monocytes (MPs), erythrocytes (EPs), and megakaryocytes (MkPs).
Growth factors are required for the survival and proliferation of hematopoietic cells at all stages of development (see figure below). Of the factors that affect multipotential cells, eryhthropoietin (EPO), thrombopoietin (TPO), steel factor, Fms-like tyrosine kinase 3 (FLT3) ligand, granulocyte‑macrophage colonystimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-3 (IL-3), and interleukin-7 (IL-7) are the best characterized. Each of these proteins supports the survival and proliferation of a number of distinct target cells. Collectively, these growth factors are termed **hematopoietic growth factors**.
In the figure below, cytokines and growth factors that support the survival, proliferation, or differentiation of each type of cell are shown in red. For

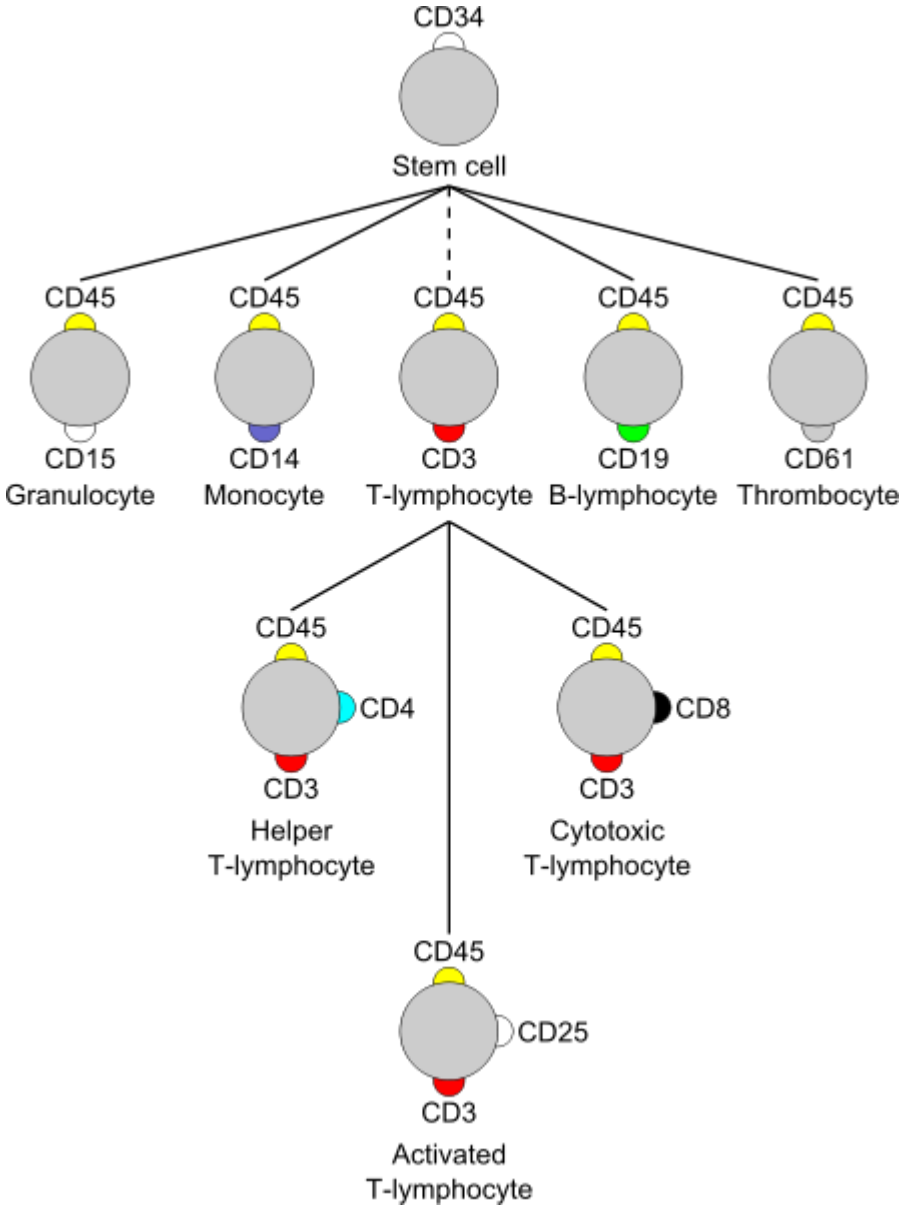
simplicity, the three types of granulocyte progenitor cells are not shown; in reality, distinct progenitors of neutrophils, eosinophils, and basophils or mast cells exist and are supported by distinct transcription factors and cytokines (e.g., interleukin-5 in the case of eosinophils, stem-cell factor (SCF) in the case of basophils or mast cells, and G-CSF in the case of neutrophils).

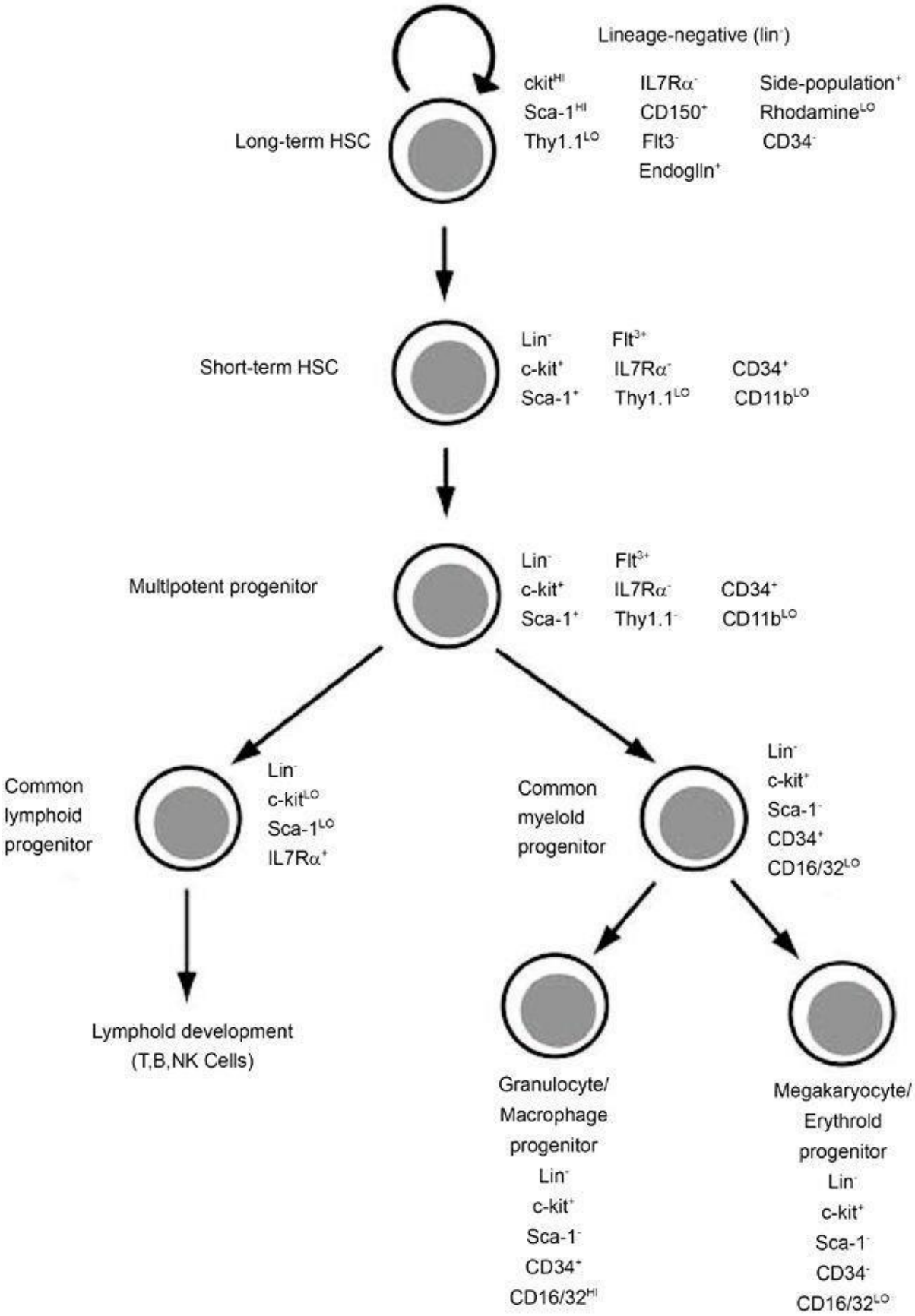



Hematopoietic Stem Cell
Self-renewal
IL-3, GM-CSF
IL-3
Myeloid Stem Cell
Lymphoid Stem Cell
SCF
Natural Killer Precursor
SCF
IL-2
Natural Killer Cell
BFU-E
CFU-GM [or CFU-C]
CFU-Eo
CFU-Bas
CFU-Mast [or CFU-Mc]
IL-1
IL-6
IL-7
SCF
IL-1
IL-2
IL-6
IL-7
SCF
IL-3
Epo
GM-CSF
IL-3
GM-CSF
IL-3
GM-CSF
G-CSF
IL-3
IL-5
GM-CSF
IL-4
IL-3
IL-4
GM-CSF
SCF
IL-3
B-Cell Progenitor
TCRγ/δ
CD3
T-Cell [TCRγ/δ+]
T-Cell Progenitor [CD4-CD8-TCR-]
CFU-E
CFU-Meg [or CFU-Mk]
CFU-G
CFU-M
Myeloblast
Myeloblast
Mast Cell
IL-6
IL-7
IL-11
IL-12
LIF
G-CSF
SCF
IL-3
IL-2
IL-4
IL-7
IL-9
IL-10
Proery-throblast
Megakarya-blast
Myeloblast
Monoblast
Pro B-Cell [Germ-line VDJ]
TCRα/β
CD4
CD8
T-Cell [CD4+CD8+TCRα/β+]
Eosinophilic Myelocyte
Basophilic Myelocyte
IL-1, IL-2
IL-3, IL-4
IL-5, IL-6
IL-7
T-Memory Cell
Neutrophilic Myelocyte
Promonocyte
IgM/
IgD
Mature B-Cell
Erythrocytes
Eosinophil
Basophil
IL-2
IL-7
IL-12
IL-2
IL-5
IL-7
IL-12
TCRα/β
CD4
CD3
TCRα/β
CD8
CD3
Monocyte
Megakaryocyte
Switched Plasma Cell Secreting non-IgM
IgM Antibody Secreting B-Cell
Memory B-Cell Activated upon Antigen Stimulation
TH-Cell [T-Helper Cell]
TC-Cell [Cytotoxic T-Cell]
Tpo?
IL-6
IL-8
GM-CSF
M-CSF
IL-3
T-Helper Differentiation
Polymorpho-nucleated Neutrophil
Platelets [or Thrombocytes]
IgE
IgA
IgG
IgD
IgM
T-Helper Cell Sub-sets
TH1
Treg
TH2
TH17
[CD4+CD25+FOXP3]
Macrophage
Dendritic Cell

Immunophenotype

The cluster of differentiation (CD) molecules are cell surface markers useful for the identification and characterization of leukocytes. The CD system is commonly used as cell markers in immunophenotyping, allowing cells to be defined based on what molecules are present on their surface. While using one CD molecule to define populations is uncommon (though a few examples exist), combining markers has allowed for cell types with very specific definitions within the immune system.




Lineage-negative (lin-)
ckit^HI
Sca-1^HI
Thy1.1^LO
IL7Rα-
CD150+
Flt3-
EndoglIn+
Side-population+
Rhodamine^LO
CD34-
Long-term HSC
Short-term HSC
Lin-
c-kit+
Sca-1+
Flt3+
IL7Rα-
Thy1.1^LO
CD34+
CD11b^LO
Multipotent progenitor
Lin-
c-kit+
Sca-1+
Flt3+
IL7Rα-
Thy1.1-
CD34+
CD11b^LO
Common lymphoid progenitor
Lin-
c-kit^LO
Sca-1^LO
IL7Rα+
Common myelold progenitor
Lin-
c-kit+
Sca-1-
CD34+
CD16/32^LO
Lymphold development
(T,B,NK Cells)
Granulocyte/ Macrophage progenitor
Lin-
c-kit+
Sca-1-
CD34+
CD16/32^HI
Megakaryocyte/ Erythrold progenitor
Lin-
c-kit+
Sca-1-
CD34-
CD16/32^LO

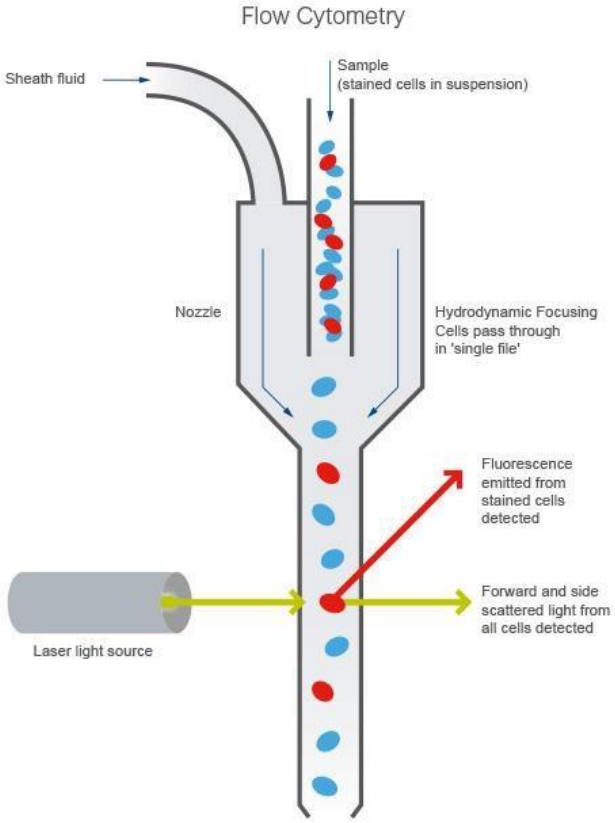
Cell	Main markers
Hematopoietic stem cells	CD34+, CD31-
Granulocyte	CD45+, CD33+, CD15+
Monocyte	CD45+, CD33+, CD14+
T lymphocyte	CD45+, CD3+
T helper lymphocyte	CD45+, CD3+, CD4+
T cytotoxic lymphocyte	CD45+, CD3+, CD8+
T regulatory lymphocyte	CD45+, CD3+, FOXP3
B lymphocyte progenitor	CD45+, CD19+
B lymphocyte	CD45+, CD20+
Natural killer cell	CD45+,CD16+,CD56+, CD3-

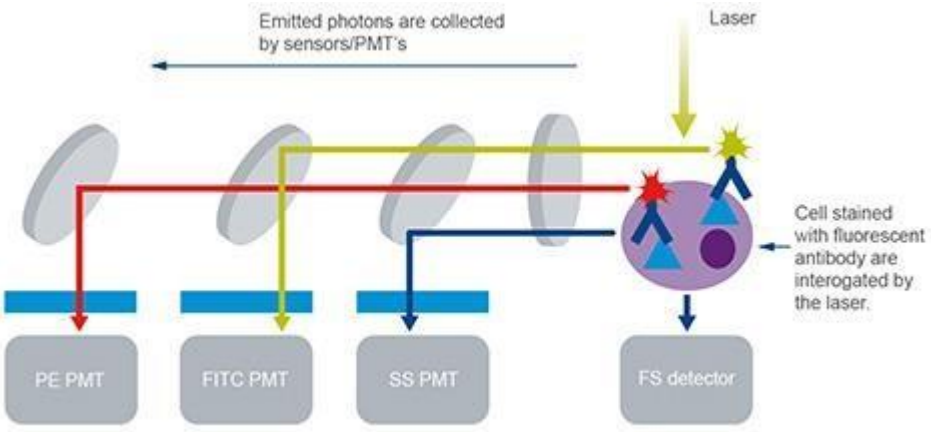
Flow Cytometry

Flow cytometry is a popular laser-based technology to analyze the characteristics of cells or particles. It is predominantly used to measure fluorescence intensity produced by fluorescent-labeled antibodies detecting proteins, or ligands that bind to specific cell-associated molecules.

When a cell suspension is run through the cytometer, sheath fluid is used to hydrodynamically focus the cell suspension through a small nozzle. The tiny stream of fluid takes the cells past the laser light one cell at a time**.** Light scattered from the cells or particles is detected as they go through the laser beam. A detector in front of the light beam measures forward scatter (FS) and several detectors to the side measure side scatter (SS). Fluorescence detectors measure the fluorescence emitted from positively stained cells or particles. Cells or particles passing through the beam scatter light, which is detected as FS and SS. FS correlates with cell size and SS is proportional to the granularity of the

cells. In this manner, cell populations can often be distinguished based on differences in their size and granularity alone.

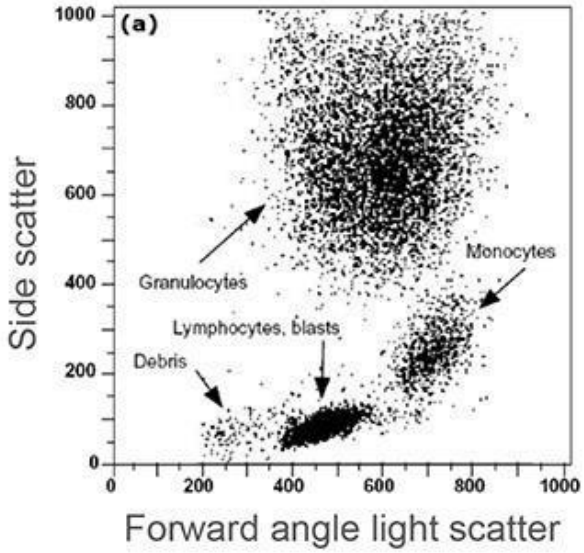




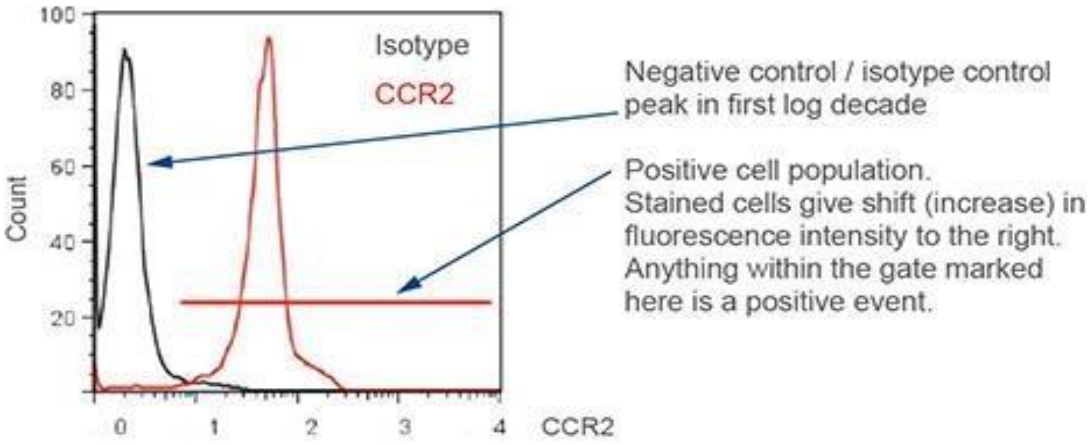
Example_1 (in the lab)

A useful example of flow cytometry is when running blood samples on the flow cytometer.

- Larger and more granular granulocyte cells produce a large population with high SS and FS.
- Monocytes are large cells, but not so granular, so these produce a separate population with high FS but lower SS.
- Smaller lymphocytes and lymphoblasts produce a separate population with less FS. They are not granular cells, so also have low SS.
  Therefore, these cells can be separated into different populations based on their FS and SS alone.



Example_2 (in the lab)



Anti-CCR2 antibody staining of human peripheral blood mononuclear cells (PBMC) gated on monocytes

Extra Example