

Med Chem 535P – Diagnostic Medicinal Chemistry
Hematology ~ Leukocytes (White Blood Cells, WBC)

I. Cell Types and Characteristics

A. Granulocytes

1. *Neutrophils (Polys or PMNs)*
2. *Eosinophils (Eosins)*
3. *Basophils (Basos)*

B. Monocytes/Macrophages

C. Lymphocytes (Lymphs)

1. *T Lymphocytes*
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III. WBC Disorders

- A. Granulocyte Disorders
- B. Lymphocyte Disorders

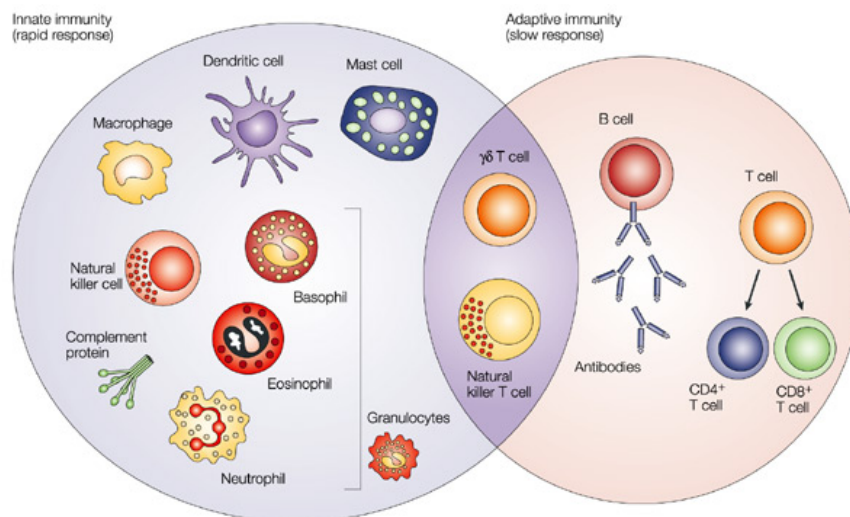
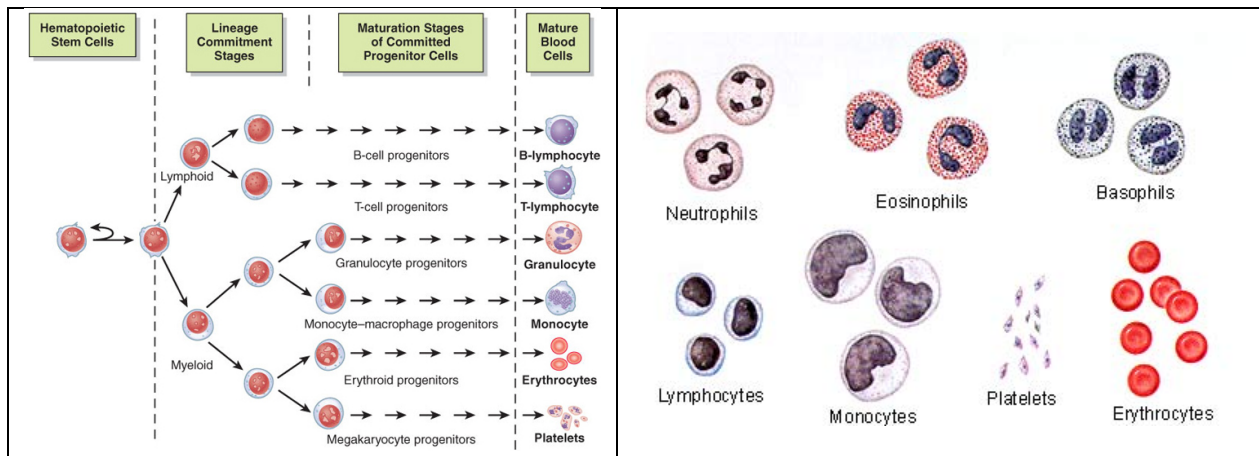
HEMATOLOGY ~ WHITE BLOOD CELLS

Leukocytes, a.k.a. White Blood Cells ~ Major function is to fight infections. Also involved in the inflammatory response and allergic reactions.

Leukocytes fall into two broad categories:

granulocytes (a.k.a., phagocytes): neutrophils, eosinophils, basophils

lymphocytes: T-lymphocytes (T-cells), B-lymphocytes (B-cells) and killer T-cells



Nature Reviews | Cancer

Innate vs. Adaptive Immune Response

I. Cell Types and Characteristics

A. **Granulocytes** represent 50-75% of all white blood cells; a.k.a., polymorphonuclear leukocytes (PMNs, polys). These cells are part of the *innate immune system*.

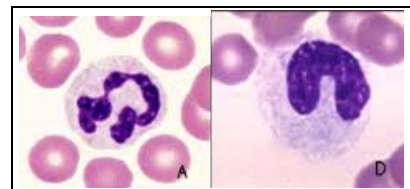
There are three subtypes that are differentiated microscopically using Wright's stain (contains acidic and basic dyes).

1. Neutrophils.

Normal Range, Segmented: 45% - 73%

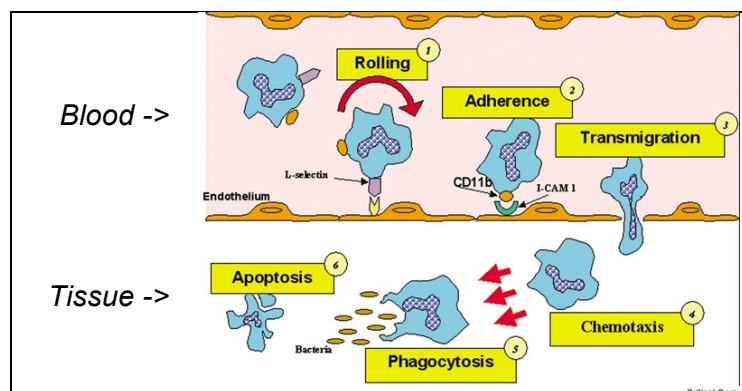
Bands: 3% - 5%

These cells do not stain well with Wright's stain and are considered *neutral*.



Bands are immature neutrophils that have been recently released into the blood. They differ from mature neutrophils in that they are unsegmented.

The circulation time for PMNs is 6 – 8 hours at which point they enter the tissues (*margination*). They undergo apoptosis unless they engage a foreign particle, specifically bacteria and fungi.



Neutrophils are attracted to the site of infection by *cytokines* that are released by activated cells ~ endothelial, mast, and macrophage cells; *chemotaxis*).

They phagocytize “opsonized” microorganisms and digest the particle within a phago-lysosome. Secretion of these degrading enzymes can lead to local tissue injury.

In addition to phagocytosis, neutrophils also release cytokines, which amplify the response.

These cells are the first defense in combating bacterial and fungal infections. 90% are stored in the bone marrow and there is an increase in circulating neutrophils in response to infection and trauma (*neutrophilia*). There is also an increase in the percentage of bands (***shift to the left***).

Absolute Neutrophil Count (ANC) ~ neutrophil absolute number vs. relative %

$$ANC = (\% \text{ neutrophils}/100 + \% \text{ bands}/100) \times (\text{WBC})$$

Note that the absolute count can be determined for any blood cell in an analogous manner. Also note that an increase in the *percentage* of neutrophils by necessity decreases the percentage of lymphocytes. This does *not* constitute a lymphopenia (*percent vs. absolute counts*).

2. Eosinophils (Eosins). *Normal Range: 0 – 4%*.

These granulocytes contain basic proteins that stain with the red acidic dye (eosin).

Eosinophils primarily reside in the intestine and lungs and have limited phagocytic capacity. Their primary role is in killing larger parasites that cannot be phagocytized (e.g., enteric nematodes). They recognize the Fc portion of IgE antibodies bound to the parasite, which triggers degranulation and release of compounds that are toxic to both parasite and host tissues (peroxidases, nucleases, histamine, etc.).

Eosinophils are attracted to the site of an allergic reaction by mast cell degranulation (histamine, heparin, eosinophil chemotactic factor). They thus play a role in modulation of the allergic inflammation, rhinitis, and asthma.

3. Basophils (Basos). *Normal Range: 0 – 1%*

These granulocytes contain acidic proteins that stain with the blue/purple basic dye (methylene blue). They are involved in inflammatory and allergic responses.

When stimulated by a foreign pathogen, basophils and mast cells (tissue basos?) secrete inflammatory mediators, including histamine and heparin to mediate the inflammatory response (increased blood flow and permeability of the tissues).

Basos are also associated with allergic responses and *basophilia* can be observed in cases of chronic inflammation.

Granulocytes die in the course of destroying the ingested particles yielding pus.

B. **Monocytes/Macrophages**. *Normal Range: 2 – 8%*

Monocytes leave the circulation, attracted by cytokines, and enter the tissues where they mature into *macrophages*. They are present in skin (Langerhans cells) lymph nodes, alveoli (dust cells), spleen (sinusoidal cells), liver (Kupffer cells), and bone marrow (osteoclasts).

They play a major role in removing cellular “aged” neutrophils, cellular debris, pathogens, and in the destruction of damaged erythrocytes, plasma proteins, and plasma lipids. They also play a role in tumor cell cytotoxicity.

Macrophages can reside in tissues, actively phagocytosing material for months.

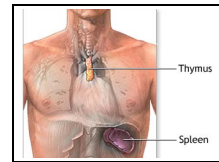
Macrophages can recognize and phagocytize pathogens. They digest the particle and “present” peptides on their surface (antigen presenting cell). The peptide is recognized by a T-lymphocyte, which is then activated.

C. **Lymphocytes** (Lymphs). *Normal Range: 20 – 40%*

Lymphocytes make up the second major group of leukocytes and are primarily involved in immune reactions. They form the cellular components of the *adaptive immune system*.

These cells are *non-phagocytic*. They are difficult to differentiate visually but can be differentiated by the presence of specific surface protein known as *clusters of differentiation* (CD). This is done by *fluorescence-activated cell sorting* (FACS).

1. T Lymphocytes (CD3) are the major circulating lymphocytes (~ 75%). They are released from the bone marrow and mature in the thymus.



They are responsible for *cell-mediated immunity*, play a role in delayed hypersensitivity reactions (TB, mumps tests) and also in organ transplant rejection. They are specifically tailored to defend against intracellular pathogens (e.g., viruses).

- CD4⁺ *helper T cells* produce cytokines that activate macrophages, B cells, and NK cells.
- CD8⁺ *cytotoxic T cells* bind to virus-infected and/or damaged cells and release cytotoxins that kill the cell by lysis and/or apoptosis.

They also play an important role in killing tumor cells.

The HIV virus binds to the CD4 receptor and infects the helper cell but does not elicit an antiviral response. The cells are destroyed and the CD4/CD8 ratio decreases. Quantitation of CD4 T-cell count and viral load (by PCR) are used to monitor infection prognosis.

T lymphocytes are the primary mediator of tissue rejection. Anti-T cell therapies include corticosteroids and anti-CD3 antibodies (Muromonab).

2. B Lymphocyte (CD20) fully mature in the bone marrow. When stimulated by antigen-presenting cells (APCs; activated T-cells, dendritic cells), they mature into *plasma cells* that secrete antibodies that neutralize the antigenic particle (virus, bacteria) or target it for *opsonization*. They neutralize pathogens prior to entry into the cell.

This is the *humoral branch* of the adaptive immune system.

3. Natural Killer (NK) cells (CD 56) are actually part of the innate immune system because they do not require antigen presentation to initiate a response. Upon target recognition, they secrete cytotoxic compounds that can lyse the cell and/or induce apoptosis.

NK cells play an important role in the destruction of virally-infected cells and tumor cells.

II. Lab Tests

A. White Blood Cell Count. *Normal Range: $3.8 - 9.8 \times 10^3$ cells/ μ l (100%)**

B. Differential Count (Diff)* is the proportion of the various WBC types. May be presented as absolute numbers or as a % of total

WBC	%	Cells/ μ l
Total	100	$4.4 - 11.3 \times 10^3$
PMN Neutrophils	45 - 73	2,000 - 8,300
Bands	3 - 5	130 - 570
Eosinophils	0 - 4	0 - 450
Basophils	0 - 1	0 - 200
Lymphocytes	20 - 40	900 - 4,500
Monocytes	2 - 8	90 - 800

ANC ~ Absolute Neutrophil Count (includes immature cells)

ALC ~ Absolute Lymphocyte Count

Both of the above values are calculated by multiplying the CBC by the Diff %

“*Shift to the Left*” means that there is a shift in the WBC towards more immature cells (more bands and blasts). This is observed in most bacterial infections and in some cancers.

“*Shift to the Right*” means that there has been a shift back to the normal Diff.

III. WBC Disorders

General Terms: *Aplastic anemia* ~ loss of all blood cell types
 Leukopenia ~ a decrease in the WBC count
 Leukocytosis ~ an increase in the WBC count
 Agranulocytosis ~ a loss of granulocytes

A. Granulocyte Disorders

1. Neutrophils

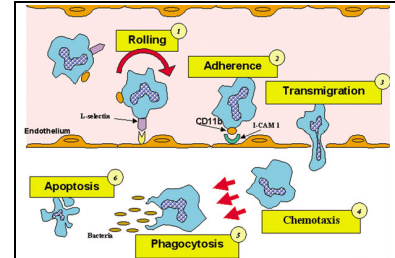
i. Neutrophilia

- A neutrophil count of over 9,000 cells/ μ l and up to 30,000 cells/ μ l with a shift to the left typically indicates a bacterial infection, but can also be seen with tissue trauma (burns, necrosis, MI) and with severe inflammatory disease (e.g., rheumatoid arthritis).
- A neutrophil count of over 100,000 cells/ μ l is observed in the active phase of Acute Myeloblastic Anemia (AML) and Chronic Myelocytic Leukemia (CML) along with major shifts to the left.

Drugs: **Lithium** can cause a modest increase in neutrophils. **Glucocorticoids, adrenaline** and exercise can lead to neutrophilia without a shift to the left. This is primarily due to “*demargination*”.

Note: Steroids are used to treat a variety of immune and inflammatory disorders and yet they *increase* WBC. This is because steroids affect WBC *function*.

1. Steroids interfere with production of cytokines and thus the proliferation and interaction of *T cells*.
2. Steroids interfere with the binding of interleukins to *B cells*, which means that the B cells have a hard time proliferating and making antibodies.
3. Steroids inhibit just about everything that *neutrophils* do: adhesion, chemotaxis, phagocytosis, and the release of toxic substances.
4. Steroids down-regulate the expression of Fc receptors on *macrophages* – so they are less able to phagocytose opsonized things.



ii. Neutropenia

- A neutrophil count *less than 1,500 cells/ μ l* can occur with acute overwhelming bacterial infections.
- Severe neutropenia is also observed secondary to bone marrow damage resulting from **radiation therapy, antineoplastic agents**, and some antibiotics.

Drugs that can cause neutropenia include penicillins, **chloramphenicol**, ganciclovir and phenothiazines.

A neutrophil count < 500 cells/ μ l is associated with spontaneous bacterial/fungal infections; agranulocytosis: *neutrophil count < 100 cells/ μ l*

Lithium and recombinant Granulocyte Colony-Stimulating Factor (G-CSF) can be used to treat neutropenia.

2. *Eosinophils*

- i. Eosinophilia is characterized by eosinophil counts > 450 cells/ μ l (Diff $> 5\%$). This is observed with **parasitic infections**, **allergic disorders** (asthma), chronic disease (rheumatoid arthritis), and malignant diseases (Hogkin's disease).

This can also occur with some drugs: ACE inhibitors, **sulfonamides**, tetracyclines, penicillins, cephalosporins, phenothiazines, NSAIDs, among others.

- ii. Eosinopenia is rare but sometimes observed with acute infections and after epinephrine injections and high dose corticosteroid therapy.

3. *Basophilia* is associated with myeloproliferative disorders and inflammatory reactions and diseases.

B. Lymphocyte Disorders

1. *Lymphocytosis*: $> 4,500$ cells/ μ l

- i. There is a *relative* increase in neutropenic conditions (% Diff)
- ii. Absolute increases are observed with many **viral infections** (CMV, EBV, varicella)
- iii. Absolute increases also occur in Acute Lymphoblastic **Leukemia** (ALL) and in Chronic Lymphocytic Leukemia (CLL).

Chronic narcotic use can cause lymphocytosis

2. *Lymphopenia*: < 900 cells/ μ l

- i. There is a *relative* decrease in neutrophilic conditions (% Diff)
- ii. An absolute decrease occurs with immunodeficiency states ~ **AIDS**, Hodgkin's lymphoma, and **radiation therapy**.

High-dose corticosteroid therapy can lead to lymphopenia.

Hematology, WBC Study Guide

Terms You Need to Know

Agranulocytosis
Aplastic anemia
Chemotaxis
Cytokine
Margination
Opsonization

You should be prepared to discuss the major white blood cell types as presented in class and in the notes. What is the difference between granulocytes and lymphocytes and which cells belong to each class. How are they differentiated?

You should be prepared to describe the difference between the innate and adaptive immune systems. Which cells belong to each system?

You should be prepared to discuss the primary function of each white blood cell. Which cells are phagocytic? Which cells product antibodies? What is the primary biological role for each cell type?

You should be prepared to provide the relative abundance of each cell type under normal physiological conditions. How are they reported in a CBC with differential? How are they quantified?

You should be prepared to discuss the difference between a relative and absolute cell count. Why is this important? Be prepared to calculate ANC and ALC from a CBC.

You should be prepared to describe clinical conditions that are associated with an increase in specific cell types (i.e., bacterial infection, fungal infection, parasitic infection, viral infection, allergic response, inflammation, etc.). What is the significance of pus.

You should be prepared to discuss factors that can result in neutrophilia and neutropenia, as discussed in class and in the notes. What drugs are associated with these conditions?

You should be prepared to discuss the significance of a shift to the left and the relationship with bands.

Be prepared to describe margination and de-margination. What is the significance of neutrophilia associated with corticosteroid administration? What is the effect on bands, and why? Do increased numbers mean increased function?

Be prepared to discuss any drug colored in red in any of the lecture notes ...