Blood

Blood is a liquid tissue. Suspended in the watery **plasma** are seven types of cells and cell fragments.

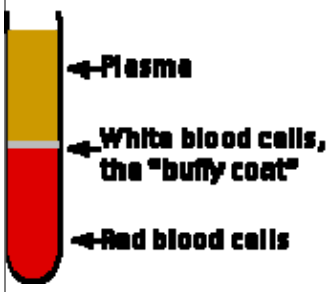
- **red blood cells** (**RBC**s) or **erythrocytes**
- **platelets** or **thrombocytes**
- five kinds of **white blood cells** (**WBC**s) or **leukocytes**
    - Three kinds of **granulocytes**
        - **neutrophils**
        - **eosinophils**
        - **basophils**
    - Two kinds of leukocytes without granules in their cytoplasm
        - **lymphocytes**
        - **monocytes**

Index to this page

If one takes a sample of blood, treats it with an agent to prevent clotting, and spins it in a centrifuge,

- the red cells settle to the bottom
- the white cells settle on top of them forming the "buffy coat".

The fraction occupied by the red cells is called the **hematocrit**. Normally it is approximately 45%. Values much lower than this are a sign of anemia.



Functions of the blood

Blood performs two major functions:

- transport through the body of

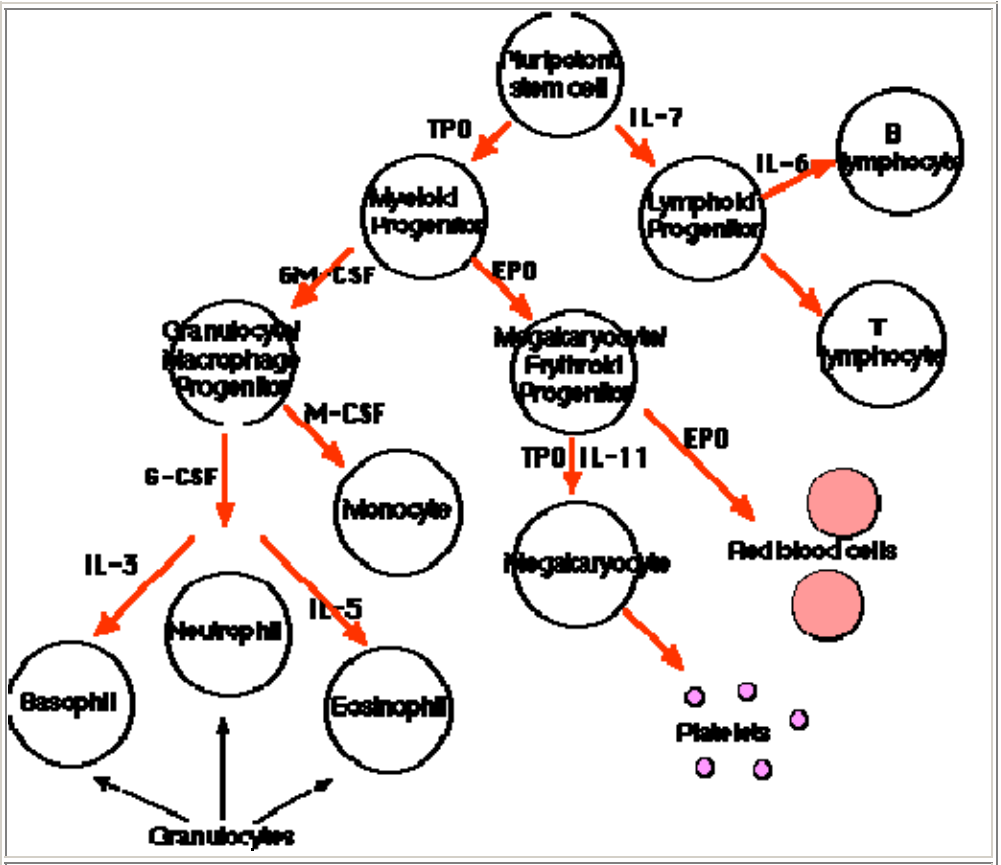
- oxygen and carbon dioxide
- food molecules (glucose, lipids, amino acids)
- ions (e.g., Na^+, Ca^{2+}, HCO_3^-)
- wastes (e.g., urea)
- hormones
- heat

- defense of the body against infections and other foreign materials. All the WBCs participate in these defenses.

The formation of blood cells (cell types and acronyms are defined below)

All the various types of blood cells

- are produced in the **bone marrow** (some 10^{11} of them each day in an adult human!).
- arise from a single type of cell called a **pluripotent stem cell**.



Link to a diagram showing the actual appearance of these cells (96K)

These stem cells

- are very rare (only about one in 10,000 bone marrow cells)
- express a surface protein designated CD34
- produce, by mitosis, two kinds of progeny
    - more stem cells
    - cells that begin to differentiate along the paths leading to the various kinds of blood cells.

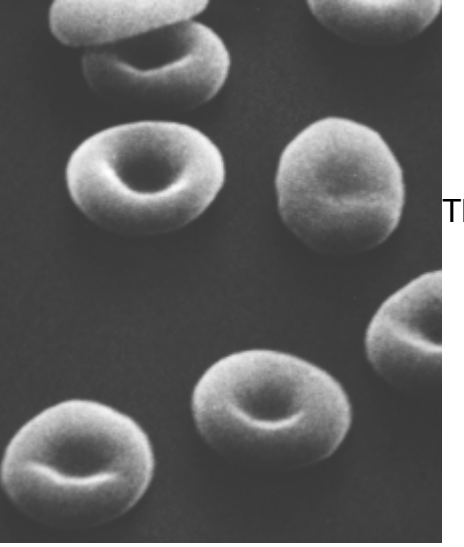
Which path is taken is regulated by

- the need for more of that type of blood cell which is, in turn, controlled by appropriate cytokines and/or hormones.

Examples:

- **lymphocytes**. Interleukin-7 (**IL-7**) is a major factor in stimulating bone marrow stem cells to start down the path leading to the various lymphocytes (mostly B cells and T cells).
- The hormone **thrombopoietin** (**TPO**), secreted by the liver, starts cells down the path leading to **megakaryocytes** and **red blood cells**.
- **red blood cells**. **Erythropoietin** (**EPO**), produced by the kidneys, enhances the production of RBCs.
- **platelets. Thrombopoietin** (**TPO**), assisted by Interleukin-11 (**IL-11**), stimulates the production of **megakaryocytes** and their fragmentation into platelets.
- **Granulocyte-monocyte colony-stimulating factor** (**GM-CSF**), as its name suggests, sends cells down the path leading to both those cell types. In due course, one path or the other is taken.
    - Under the influence of **granulocyte colony-stimulating factor** (**G-CSF**), they differentiate into **neutrophils**.
    - Further stimulated by interleukin-5 (**IL-5**) they develop into **eosinophils**.
    - Interleukin-3 (**IL-3**) participates in the differentiation of most of the white blood cells but plays a particularly prominent role in the formation of **basophils** (responsible for some allergies).
    - Stimulated by **macrophage colony-stimulating factor** (**M-CSF**) the granulocyte/macrophage progenitor cells differentiate into **monocytes**, the precursors of **macrophages**.

Red Blood Cells (erythrocytes)

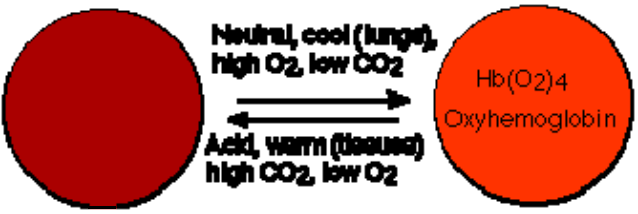


The most numerous type in the blood.

- Women average about 4.8 million of these cells per cubic millimeter (mm^3; which is the same as a microliter [µl]) of blood
- Men average about 5.4 x 10^6 per µl
- These values can vary over quite a range depending on such factors as health, and altitude. (Peruvians living at 18,000 feet may have as many as 8.3 x 10^6 RBCs per µl.)

As RBC precursors mature in the bone marrow,

- they manufacture hemoglobin until it accounts for some 90% of the dry weight of the cell.
- The nucleus is squeezed out of the cell. Nearby macrophages ingest the extruded nuclei and break down their DNA.



This scanning electron micrograph (courtesy of Dr. Marion J. Barnhart) shows the characteristic biconcave shape of red blood cells.

Thus RBCs are terminally differentiated; that is, they can never divide. They live about 120 days and then are ingested by phagocytic cells in the liver and spleen. Most of the iron in their hemoglobin is reclaimed for reuse. The remainder of the heme portion of the molecule is degraded into bile pigments and excreted by the liver. Some 3 million RBCs die and are scavenged by the liver each second.

Red blood cells are responsible for the transport of **oxygen** and **carbon dioxide**.

Oxygen Transport

The hemoglobin (Hb) molecule

- consists of four polypeptides.
- Each of these is attached the prosthetic group **heme**.
- There is one atom of iron at the center of each heme.
- One molecule of oxygen can bind to each heme.

The reaction is reversible.

- Under the conditions of lower temperature, higher pH, and increased oxygen pressure in the capillaries of the lungs, the reaction proceeds to the right. The purple-red deoxygenated hemoglobin of the venous blood becomes the bright-red **oxyhemoglobin** of the arterial blood.

- Under the conditions of higher temperature, lower pH, and lower oxygen pressure in the tissues, the reverse reaction is promoted and oxyhemoglobin gives up its oxygen.

Carbon Dioxide Transport

Carbon dioxide (CO_2) combines with water forming carbonic acid, which dissociates into a hydrogen ion (H^+) and a bicarbonate ions:

$$CO_2 + H_2O <-> H_2CO_3 <-> H^+ + HCO_3^-$$

95% of the CO_2 generated in the tissues is carried in the red blood cells:

- About one-half of this is directly bound to hemoglobin (at a site different from the one that binds oxygen).
- The rest is converted - following the equation above - by the enzyme **carbonic anhydrase** into
    - bicarbonate ions that diffuse back out into the plasma and
    - hydrogen ions (H^+) that bind to the protein portion of the hemoglobin (thus having no effect on pH).

Only about 5% of the CO_2 generated in the tissues dissolves directly in the plasma. (A good thing, too: if all the CO_2 we make were carried this way, the pH of the blood would drop from its normal 7.4 to an instantly-fatal 4.5!)

When the red cells reach the lungs, these reactions are reversed and CO_2 is released to the air of the alveoli.

Anemia

Anemia is a shortage of

- RBCs and/or
- the amount of hemoglobin in them.

Anemia has many causes. One of the most common is an inadequate intake of iron in the diet.

Blood Groups

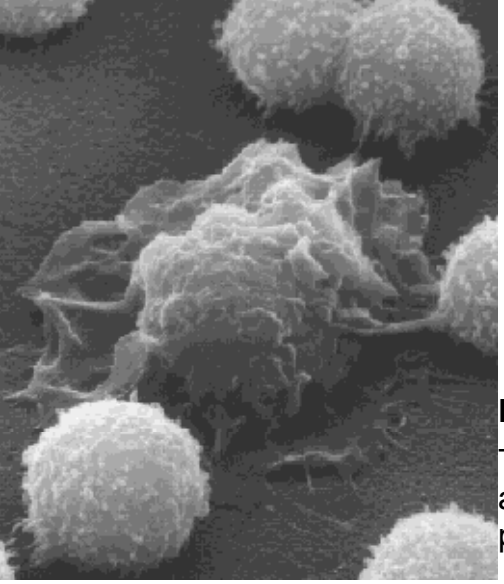
Red blood cells have surface antigens that differ between people and that create the so-called blood groups such as the **ABO** system.

Link to a discussion of blood groups.

White Blood Cells (leukocytes)

White blood cells

- are much less numerous than red (the ratio between the two is around 1:700),
- have nuclei,



- participate in protecting the body from infection,
- consist of **lymphocytes** and **monocytes** with relatively clear cytoplasm, and three types of **granulocytes**, whose cytoplasm is filled with granules.

Lymphocytes

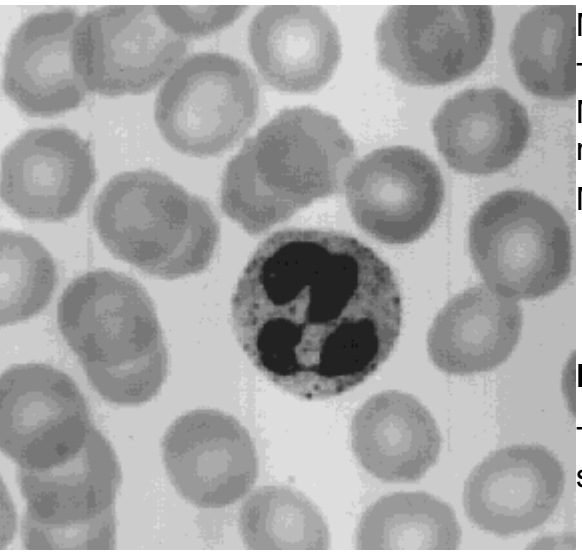
There are several kinds of lymphocytes (although they all look alike under the microscope), each with different functions to perform . The most common types of lymphocytes are

- **B lymphocytes** ("B cells"). These are responsible for making antibodies.
- **T lymphocytes** ("T cells"). There are several subsets of these:
  - **inflammatory T cells** that recruit macrophages and neutrophils to the site of infection or other tissue damage
  - **cytotoxic T lymphocytes** (CTLs) that kill virus-infected and, perhaps, tumor cells
  - **helper T cells** that enhance the production of antibodies by B cells

Although bone marrow is the ultimate source of lymphocytes, the lymphocytes that will become T cells migrate from the bone marrow to the thymus where they mature. Both B cells and T cells also take up residence in lymph nodes, the spleen and other tissues where they

- encounter antigens
- continue to divide by mitosis
- mature into fully functional cells.

Monocytes



Monocytes leave the blood and become **macrophages**.

This scanning electron micrograph (courtesy of Drs. Jan M. Orenstein and Emma Shelton) shows a single macrophage surrounded by several lymphocytes.

Macrophages are large, phagocytic cells that engulf

- foreign material (antigens) that enter the body
- dead and dying cells of the body.

Neutrophils

The most abundant of the WBCs. This photomicrograph shows a single neutrophil surrounded by red blood cells.

Neutrophils squeeze through the capillary walls and into infected tissue where they kill the invaders (e.g., bacteria) and then engulf the remnants by phagocytosis.

This is a never-ending task, even in healthy people: Our throat, nasal passages, and colon harbor vast numbers of bacteria. Most of these are commensals, and do us no harm. But that is because neutrophils keep them in check.

However,

- heavy doses of radiation
- chemotherapy
- and many other forms of stress

can reduce the numbers of neutrophils so that formerly harmless bacteria begin to proliferate. The resulting **opportunistic infection** can be life-threatening.

Eosinophils

The number of eosinophils in the blood is normally quite low (0 - 450/µl). However, their numbers increase sharply in certain diseases, especially infections by parasitic worms. Eosinophils are cytotoxic, releasing the contents of their granules on the invader.

Basophils

The number of basophils also increases during infection. Basophils leave the blood and accumulate at the site of infection or other inflammation. There they discharge the contents of their granules, releasing a variety of mediators such as:

- histamine
- serotonin
- prostaglandins and leukotrienes

which increase the blood flow to the area and in other ways add to the inflammatory process. The mediators released by basophils also play an important part in some allergic responses such as

- hay fever and
- an anaphylactic response to insect stings.

Platelets

Platelets are cell fragments produced from **megakaryocytes**.

Blood normally contains 150,000 to 450,000 per microliter (µl). If this value should drop much below 50,000/µl, there is a danger of uncontrolled bleeding. This is because of the essential role that platelets have in blood clotting.

When blood vessels are cut or damaged, the loss of blood from the system must be stopped before shock and possible death occur. This is accomplished by solidification of the blood, a process called **coagulation** or clotting.

A blood clot consists of

- a **plug of platelets** enmeshed in a
- network of insoluble **fibrin** molecules.

Details of the clotting process are in a separate page. Link to it.

Plasma

Plasma is the straw-colored liquid in which the blood cells are suspended.

Composition of blood plasma	
Component	**Percent**
Water	~92
Proteins	6-8
Salts	0.8
Lipids	0.6
Glucose (blood sugar)	0.1

Plasma transports materials needed by cells and materials that must be removed from cells:

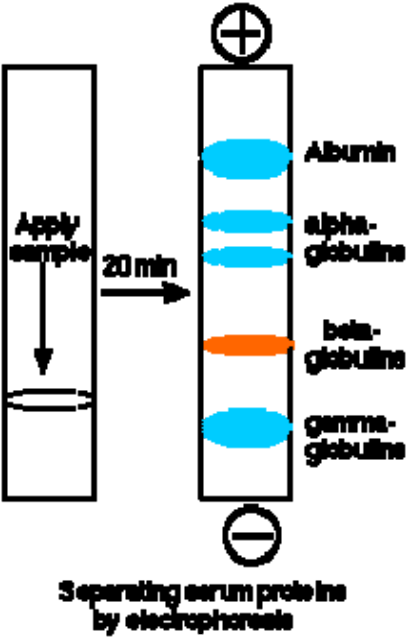
- various ions (Na^+, Ca^{2+}, HCO_3^-, etc.
- glucose and traces of other sugars
- amino acids
- other organic acids
- cholesterol and other lipids
- hormones
- urea and other wastes

Most of these materials are in transit from a place where they are added to the blood (a "source")

- exchange organs like the intestine
- depots of materials like the liver

to places ("sinks")where they will be removed from the blood.

- every cell
- exchange organs like the kidney, and skin.



Serum Proteins

Proteins make up 6-8% of the blood. They are about equally divided between **serum albumin** and a great variety of **serum globulins**.

After blood is withdrawn from a vein and allowed to clot, the clot slowly shrinks. As it does so, a clear fluid called serum is squeezed out. Thus:

Serum is blood plasma without fibrinogen and other clotting factors.

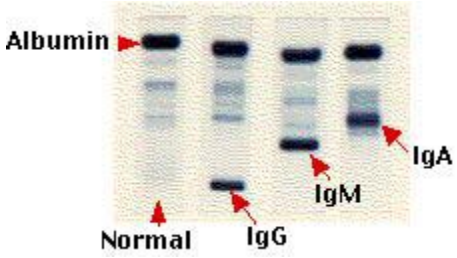
The serum proteins can be separated by **electrophoresis**.

- A drop of serum is applied in a band to a thin sheet of supporting material, like paper, that has been soaked in a slightly-alkaline salt solution.
- At pH 8.6, which is commonly used, all the proteins are negatively charged, but some more strongly than others.

Link to an explanation of how pH affects the net charge on proteins.

- A direct current can flow through the paper because of the conductivity of the buffer with which it is moistened.
- As the current flows, the serum proteins move toward the positive electrode.
- The stronger the negative charge on a protein, the faster it migrates.
- After a time (typically 20 min), the current is turned off and the proteins stained to make them visible (most are otherwise colorless).
- The separated proteins appear as distinct bands.
- The most prominent of these and the one that moves closest to the positive electrode is **serum albumin**.
- Serum albumin
    - is made in the liver
    - binds many small molecules for transport through the blood
    - helps maintain the osmotic pressure of the blood
- The other proteins are the various serum globulins.
- They migrate in the order
    - **alpha globulins** (e.g., the proteins that transport thyroxine and retinol [vitamin A])

- **beta globulins** (e.g., the iron-transporting protein **transferrin**)
- **gamma globulins**.
  - Gamma globulins are the least negatively-charged serum proteins. (They are so weakly charged, in fact, that some are swept in the flow of buffer back toward the negative electrode.)
  - Most antibodies are gamma globulins.
  - Therefore gamma globulins become more abundant following infections or immunizations.

    If an antibody-secreting cell - called a plasma cell - becomes cancerous, it grows into a clone secreting a **single kind of antibody molecule**. The image (courtesy of Beckman Instruments, Inc.) shows - from left to right - the electrophoretic separation of:

    1. **normal** human serum with its diffuse band of gamma globulins;
    2. serum from a patient with **multiple myeloma** producing an **IgG** myeloma protein;
    3. serum from a patient with Waldenström's macroglobulinemia where the cancerous clone secretes an **IgM** antibody;
    4. serum with an **IgA** myeloma protein.

    Discussion of the 5 classes of antibody molecules.
  - Gamma globulins can be harvested from donated blood (usually pooled from several thousand donors) and injected into persons exposed to certain diseases such as chicken pox and hepatitis. Because such preparations of **immune globulin** contain antibodies against most common infectious diseases, the patient gains temporary protection against the disease.

Serum Lipids

Because of their relationship to cardiovascular disease, the analysis of serum lipids has become an important health measure.

The table shows the range of typical values as well as the values above (or below) which the subject may be at increased risk of developing atherosclerosis.

LIPID	Typical values (mg/dl)	Desirable (mg/dl)
Cholesterol (total)	170-210	<200
LDL cholesterol	60-140	<130
HDL cholesterol	35-85	>35
Triglycerides	40-150	<135

- Total cholesterol is the sum of
  - HDL cholesterol
  - LDL cholesterol and
  - 20% of the triglyceride value
- Note that
  - **high LDL** values are **bad**, but
  - **high HDL** values are **good**.
- Using the various values, one can calculate a **cardiac risk ratio** = **total cholesterol** divided by **HDL cholesterol**
- A cardiac risk ratio greater than 7 is considered a warning.

More on cholesterol | How cholesterol is taken into cells.

Welcome&Next Search

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8 March 2002

Index to this page

Antigen Receptors

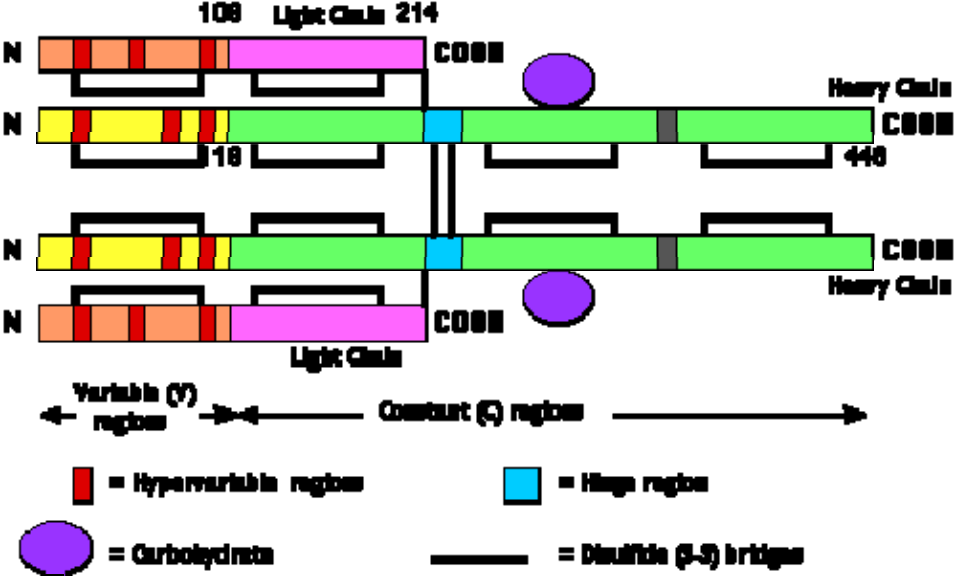
Both B cells and T cells have surface receptors for antigen. Each cell has thousands of receptors of a **single specificity**; that is, with a binding site for a particular epitope.

- T-cell receptors (**TCRs**) enable the cell to bind to and, if additional signals are present, to be activated by and respond to an epitope presented by another cell called the **antigen-presenting cell** or **APC**.
- B-cell receptors (**BCRs**) enable the cell to bind to and, if additional signals are present, to be activated by and respond to an epitope on molecules of a **soluble antigen**. The response ends with descendants of the B cell secreting vast numbers of a soluble form of its receptors. These are **antibodies**.

Discussion of antigen presentation to B cells and T cells

Antibodies

- are glycoproteins
- are built of subunits containing
    - two identical **light chains** (**L** chains), each containing about 200 amino acids
    - two identical **heavy chains** (**H** chains), which are at least twice as long as light chains
- the first 100 or so amino acids at the N-terminal of both H and L chains vary greatly from antibody to antibody
- these are the **variable (V) regions**
    - unless members of the same clone (and often not even then!), no two B cells are likely to secrete antibodies with the same variable region
    - the amino acid sequence variability in the V regions is especially pronounced in **3 hypervariable regions**
    - the tertiary structure of antibodies brings the 3 hypervariable regions of **both** the L and the H chains together
    - together they construct the **antigen binding site** against which the epitope fits
- only a few different amino acid sequences are found in the C-terminals of H and L chains
- these are the **constant (C) regions**

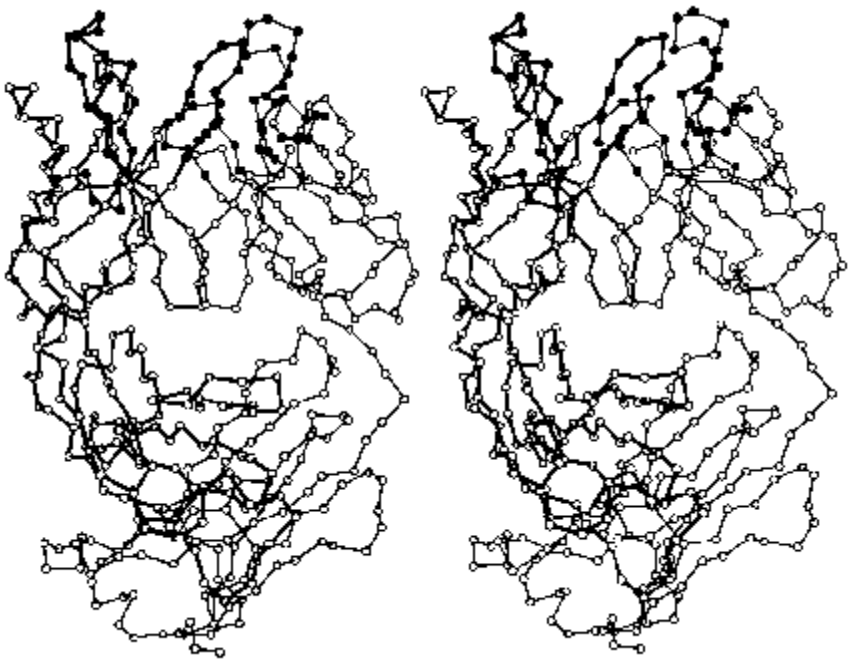


- humans make
  - two different kinds of C regions for their L chains producing
    - **kappa** (□) L chains
    - **lambda** (□) L chains
  - five different kinds of C regions for their H chains producing
    - **mu** (μ) chains (the H chain of **IgM** antibodies)
    - **gamma** (□) chains (**IgG**)
    - **alpha** (□) chains (**IgA**)
    - **delta** (□) chains (**IgD**)
    - **epsilon** (□) chains (**IgE**)
  - each of these 5 kinds of H chains seems to have no particular preference for pairing with lambda or kappa L chains

This image represents the polypeptide chain structure of a molecule of **IgG**. The numbers indicate the number of amino acids (counting from the amino terminal ("N"). In the actual molecule, the chains are folded to that each cysteine is brought close to the partner with which it forms a **disulfide** (**S-S**) **bridge**.

The images below (courtesy of Dr. D. R. Davies) represent the folded (tertiary) structure of an **entire L chain** (right side with thin connecting lines) and the **V region** plus the first third of the C region of a **heavy chain** (left side; darker lines). Each circle represents the location in 3D space of an alpha carbon. The filled circles at the top are amino acids in the **hypervariable regions**; they form the site that binds the antigen.

Do try to fuse these two images into a stereoscopic (3D) view. I find that it works best when my eyes are about 18" from the screen and I try to relax so that my eyes are directed at a point behind the screen.



Antibody molecules have two functions to perform:

- recognize and bind to an epitope on an antigen
- trigger a useful response to the antigen

The division of labor is:

- V regions are responsible for epitope recognition
- C regions are responsible for triggering a useful response

So, V regions finger the culprit; the C regions take action.

Why 5 kinds of heavy chains? To provide for different effector functions.

The 5 classes of antibodies					
Class	**H chain**	**L chain**	**Subunits**	**mg/ml**	**Notes**
IgG	gamma	kappa or lambda	H_2L_2	13	transferred across placenta
IgM	mu	kappa or lambda	$(H_2L_2)_5$	0.5-2.5	first antibodies to appear after immunization
IgA	alpha	kappa or lambda	$(H_2L_2)_2$	0.5-3	much higher concentrations in secretions
IgD	delta	kappa or lambda	H_2L_2	0.03	function uncertain

IgE	epsilon	kappa or lambda	H_2L_2	0.0003	binds to basophils and mast cells sensitizing them for certain allergic reactions

"**mg/ml**" gives the concentration normally found in human serum.
Note also that humans actually make four slightly different versions of IgG (and two of IgA); in both cases, encoded by different C-region gene segments.

If an antibody-secreting cell becomes cancerous, it will grow into a clone secreting its single class of molecule. The disease is called **multiple myeloma**. See how **IgG**, **IgM**, and **IgA** molecules appear in the serum of these patients.

T-Cell Receptors for Antigen (TCRs)

alpha/beta (□□) T cells

The antigen receptor on most T cells is made up of two transmembrane polypeptides designated **alpha** and **beta** (thus forming a heterodimer).

Like antibodies

- each has an N-terminal **variable region**
- the variable regions of the two chains cooperate to form a single binding site for the epitope

Links to more on □□ T cells:

- general discussion
- $CD4^+$ subset
- $CD8^+$ subset
- Helper subsets (Th1 and Th2)

gamma/delta (□□) T cells

A small percentage of the T cells in the blood use a TCR consisting of a heterodimer of two other types of transmembrane polypeptides: gamma and delta. The function of this subset of T cells is still a mystery. [More]

Antigen Receptor Genes

All the antibody **Heavy** chains, **lambda** L chains, **kappa** L chains, T cell **alpha**, **beta**, **gamma**, and **delta** chains are encoded by genes that are assembled from separate **gene segments** during the differentiation of the cell. The result is a single gene that is transcribed into a mRNA to be translated into one chain of the receptor. The number of gene segments from which the variable regions are constructed is sufficiently large that both B cells and T cells can generate as many as 10^{10} different antigen-binding sites. There is probably no epitope that could exist for which BCRs and TCRs able to bind it are not be built.

The mechanisms by which B cells and T cells generate receptors of such extraordinary diversity.

Welcome&Next Search

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10 December 2001

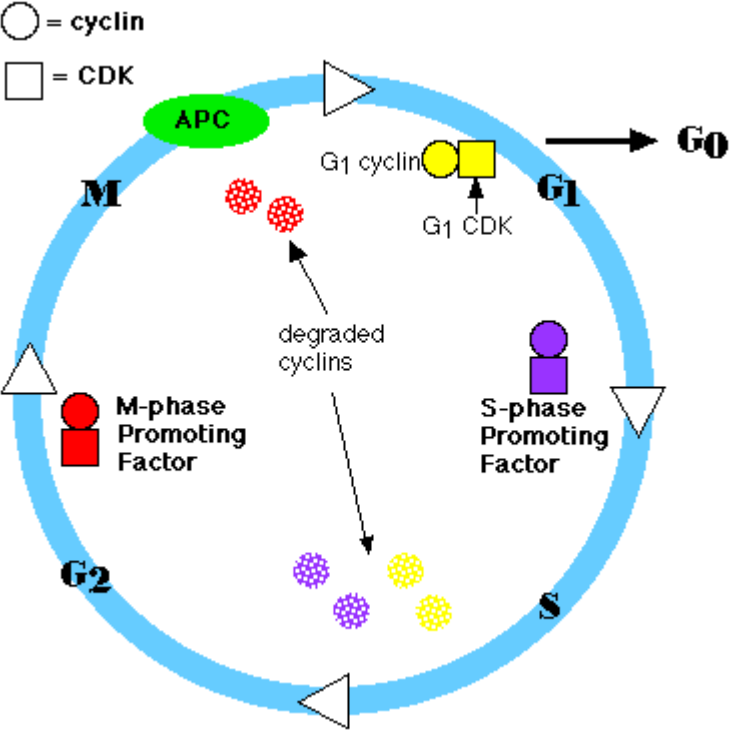
The Cell Cycle

A eukaryotic cell cannot divide into two, the two into four, etc. unless two processes alternate:

- **doubling** of its genome (**DNA**) in **S phase** (synthesis phase) of the cell cycle
- **halving** of that genome during mitosis (**M phase**)

The period between M and S is called $\mathbf{G_1}$; that between S and M is $\mathbf{G_2}$.

Index to this page



So, the cell cycle consists of:

- **G_1** = growth and preparation of the chromosomes for replication
- **S** = synthesis of DNA (and centrioles) [see DNA Replication]
- **G_2** = preparation for
- **M** = mitosis

Control of the Cell Cycle

The passage of a cell through the cell cycle is controlled by proteins in the cytoplasm. Among the main players in animal cells are:

- **Cyclins** There are 3 groups:
  - **G_1 cyclins**
  - **S-phase cyclins**
  - **M-phase cyclins**

  Their levels in the cell rise and fall with the stages of the cell cycle.
- **Cyclin-dependent kinases (CDKs)** Again, there are 3 groups:
  - **G_1 CDKs**
  - **S-phase CDKs**
  - **M-phase CDKs**

  Their levels in the cell remain fairly stable, but each must bind the appropriate cyclin (whose levels fluctuate) in order to be activated. They add phosphate groups to a variety of protein substrates that control processes in the cell cycle.
- The **anaphase-promoting complex (APC)** and other proteolytic enzymes. The APC
  - triggers the events leading to destruction of the **cohesins** and thus allowing the sister chromatids to separate.
  - degrades the mitotic (M-phase) cyclins

Steps in the cycle

- a rising level of **G_1 cyclins** signals the cell to prepare the chromosomes for replication

- a rising level of **S-phase promoting factor** (**SPF**) prepares the cell to enter S phase and duplicate its DNA (and its centrioles)
- as DNA replication continues, one of the cyclins shared by G_1 and S-phase CDKs (cyclin E) is destroyed and the level of mitotic cyclins begins to rise (in G_2)
- **M-phase promoting factor** (the complex of mitotic cyclins with M-phase CDK) initiates

  assembly of the mitotic spindle
  breakdown of the nuclear envelope
  condensation of the chromosomes
- these events take the cell to **metaphase** of mitosis
- at this point, the M-phase promoting factor activates the **anaphase promoting complex** (**APC**) which
  - allows the sister chromatids at the metaphase plate to separate and move to the poles (= anaphase), completing mitosis
  - destroys the M-phase cyclins. It does this by conjugating them with the protein **ubiquitin** which targets them for destruction by **proteasomes**.

    Link to discussion of proteasomes.
  - turns on synthesis of G_1 cyclins for the next turn of the cycle
  - degrades geminin, a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis.

    This is only one mechanism by which the cell ensures that every portion of its genome is copied once - and only once - during S phase. Link to discussion.

Some cells deliberately cut the cell cycle short allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication** and is described on a separate page. Link to it.

Checkpoints: Quality Control of the Cell Cycle

The cell has several systems for interrupting the cell cycle if something goes wrong.

- A check on completion of S phase. The cell seems to monitor the presence of the Okazaki fragments on the lagging strand during DNA replication. The cell is not permitted to proceed in the cell cycle until these have disappeared.

  Discussion of DNA replication.

- **DNA damage** checkpoints. These sense DNA damage
    - before the cell enters S phase (a G_1 checkpoint);
    - during S phase, and
    - after DNA replication (a G_2 checkpoint).
- **spindle checkpoints**. Some of these that have been discovered
    - detect any failure of spindle fibers to attach to **kinetochores** and arrest the cell in **metaphase** (M checkpoint)
    - detect improper alignment of the spindle itself and block cytokinesis

      Link to discussion of the roles of the spindle in mitosis.
    - trigger apoptosis if the damage is irreparable.

Link to description of the events in mitosis.

All the checkpoints examined require the services of a complex of proteins. Mutations in the genes encoding some of these have been associated with cancer; that is, they are oncogenes. This should not be surprising since checkpoint failures allow the cell to continue dividing despite damage to its integrity.

Examples

p53
The p53 protein senses DNA damage and can halt progression of the cell cycle in both G_1 and G_2. Both copies of the p53 gene must be mutated for this to fail so mutations in p53 are recessive, and p53 qualifies as a **tumor suppressor** gene.

Further discussion of tumor suppressor genes and p53.

The p53 protein is also a key player in **apoptosis**, forcing "bad" cells to commit suicide. So if the cell has only mutant versions of the protein, it can live on - perhaps developing into a cancer. More than half of all human cancers do, in fact, harbor p53 mutations and have no functioning p53 protein.

A genetically-engineered **adenovirus**, called **ONYX-015**, can only replicate in human cells lacking p53. Thus it infects, replicates, and ultimately kills many types of cancer cells in vitro. Clinical trials are now proceeding to see if injections of ONYX-015 can shrink a variety of types of cancers in human patients.

In some way, p53 seems to evaluate the extent of damage to DNA, at least for damage by radiation.

- At low levels of radiation, producing damage that can be repaired, p53 triggers arrest of the cell cycle until the damage is repaired.

- At high levels of radiation, producing hopelessly damaged DNA, p53 triggers apoptosis.

p53 and Aging

The protection that p53 gives us may come at a price. Genetically-engineered mice that express extrahigh levels of p53 activity gain additional protection from cancer but show many signs of premature aging (and die sooner).

ATM

ATM (="**a**taxia **t**elangiectasia **m**utated") gets its name from a human disease of that name, whose patients - among other things - are at increased risk of cancer. The ATM protein is also involved in detecting DNA damage and interrupting the cell cycle when damage is found.

MAD

MAD (="**m**itotic **a**rrest **d**eficient") encodes a protein that binds to each kinetochore **until** a spindle fiber (one microtubule will do) attaches to it. If there is any failure to attach, MAD remains and blocks entry into anaphase.

Link to discussion of chromosome behavior in anaphase.

Mutations in MAD produce a defective protein and failure of the checkpoint. The cell finishes mitosis but produces daughter cells with too many or too few chromosomes (aneuploidy). Aneuploidy is one of the hallmarks of **cancer cells** suggesting that failure of the spindle checkpoint is a major step in the conversion of a normal cell into a cancerous one.

Infection with the **human T cell leukemia virus-1** (**HTLV-1**) leads to a cancer (**ATL** = "adult T cell leukemia") in about 5% of its victims. HTLV-1 encodes a protein, called **Tax**, that binds to the MAD protein causing failure of the spindle checkpoint. The leukemic cells in these patients show many chromosome abnormalities including aneuploidy.

A **kinesin** that moves the kinetochore to the end of the spindle fiber also seems to be involved in the spindle checkpoint [More].

G_0

Many times a cell will leave the cell cycle, temporarily or permanently. It exits the cycle at G_1 and enters a stage designated G_0 (G zero). A G_0 cell is often called "quiescent", but that is probably more a reflection of the interests of the scientists studying the cell cycle than the cell itself. Many G_0 cells are anything but quiescent. They are busy carrying out their functions in the organism. e.g., secretion, conducting nerve impulses, attacking pathogens.

Often G_0 cells are terminally differentiated: they will never reenter the cell cycle but instead will carry out their function in the organism until they die.

For other cells, G_0 can be followed by reentry into the cell cycle. Most of the lymphocytes in human blood are in G_0. However, with proper stimulation, such as encountering the appropriate antigen, they can be stimulated to reenter the cell cycle (at G_1) and proceed on to new rounds of alternating **S phases** and **mitosis**.

Welcome&Next Search

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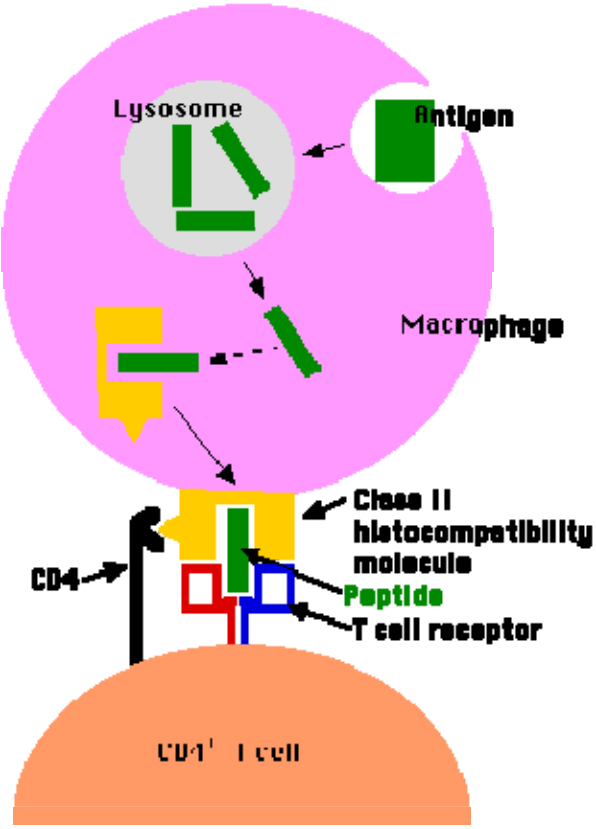
30 January 2002

Antigen Presentation



Antigens are macromolecules that elicit an immune response in the body. The most common antigens are proteins and polysaccharides. It will be helpful to distinguish between

Index to this page

- antigens that enter the body from the environment; these would include
    - inhaled macromolecules (e.g., proteins on cat hairs that can trigger an attack of asthma in susceptible people)
    - ingested macromolecules (e.g., shellfish proteins that trigger an allergic response in susceptible people)
    - molecules that are introduced beneath the skin (e.g., on a splinter or in an injected vaccine)
- antigens that are generated within the cells of the body; these would include
    - proteins encoded by the genes of viruses that have infected a cell
    - aberrant proteins that are encoded by mutant genes; such as mutated genes in cancer cells

In all cases, however, the **initial immune response** to any antigen absolutely requires that the antigen be recognized by a **T lymphocyte** ("**T cell**"). The truth of this rule is clearly demonstrated in **AIDS**: the infections (viral or fungal or bacterial) that so often claim the life of AIDS patients do so when the patient has lost virtually all of his or her $CD4^+$ T cells.

The two categories of antigens are processed and presented to T cells by quite different mechanisms.

First Group: Exogenous antigens

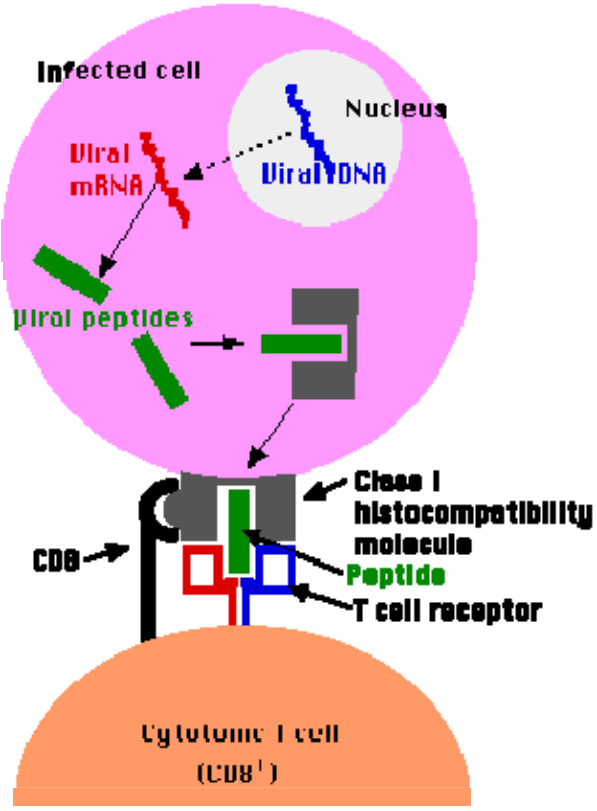
Exogenous antigens (inhaled, ingested, or injected) are taken up by "professional" antigen-presenting cells; that is, cells for which this function is their primary purpose. These include:

- **phagocytic cells** like **macrophages** and **dendritic cells**

- **B lymphocytes** ("**B cells**"); which are responsible for producing **antibodies** against the antigen.

Professional antigen-presenting cells

- engulf the antigen by endocytosis
- degrade it into fragments (e.g. break an antigenic protein into short peptides)
- display these peptides at the surface of the cell nestled within a
- **class II histocompatibility molecule**
- here they may be recognized by **CD4$^+$ T cells**



Second Group: Endogenous antigens

Antigens that are generated within a cell (e.g., viral proteins in **any** infected cell) are

- degraded into fragments (e.g., peptides) within the cell and
- displayed at the surface of the cell nestled within a
- **class I histocompatibility molecule**.
- Here they may be recognized by **CD8$^+$ T cells**.
- Most CD8$^+$ T cells are cytotoxic.
- They have the machinery to destroy the infected cell (often before it is able to release a fresh crop of viruses to spread the infection).

Now for more details.

The Class I Pathway

Class I histocompatibility molecules are transmembrane proteins expressed at the cell surface. Like all transmembrane proteins, they are synthesized by ribosomes on the rough endoplasmic reticulum (RER) and assembled within the lumen of the RER.

(See Protein Kinesis for further details.)

There are three subunits in each class I histocompatibility molecule:

- the transmembrane polypeptide (called the "heavy chain")
- the antigenic peptide
- beta-2 microglobulin

See Histocompatibility Molecules

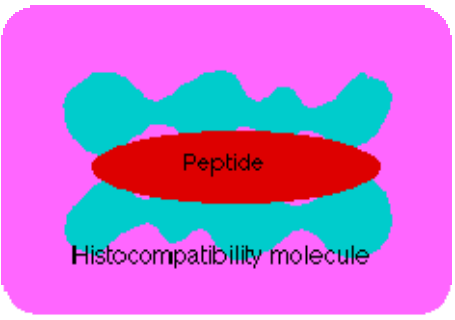
Link to model of a human class I histocompatibility molecule (92K)

All of these must be present within the lumen of the RER if they are to assemble correctly and move through the Golgi apparatus to the cell surface.

The Problem: proteins encoded by the genes of an infecting virus are synthesized in the **cytosol**. How to get them to the RER?

The Solution: **TAP** (= **t**ransporter **a**ssociated with antigen **p**rocessing).

- Viral proteins in the cytosol are degraded by **proteasomes** into viral peptides.
- The peptides are picked up by TAP proteins and transported from the cytosol into the RER where they assemble with
- the transmembrane polypeptide and beta-2 microglobulin.
- This trimolecular complex then moves through the Golgi apparatus and is inserted in the plasma membrane.
- The complex can be bound by a **T cell** with
    - a receptor (**TCR**) able to bind the peptide and flanking portions of the histocompatibility molecule (the hot dog in the bun) and
    - **CD8** molecules that bind the **CD8 receptor** (shown above as a gray hemisphere) on the histocompatibility molecule.



"Hot Dog in a Bun"
Here an antigenic peptide nestled between the alpha helices (blue) exposed at the outer surface of a histocompatibility molecule

The Class II Pathway

Class II histocompatibility molecules consist of

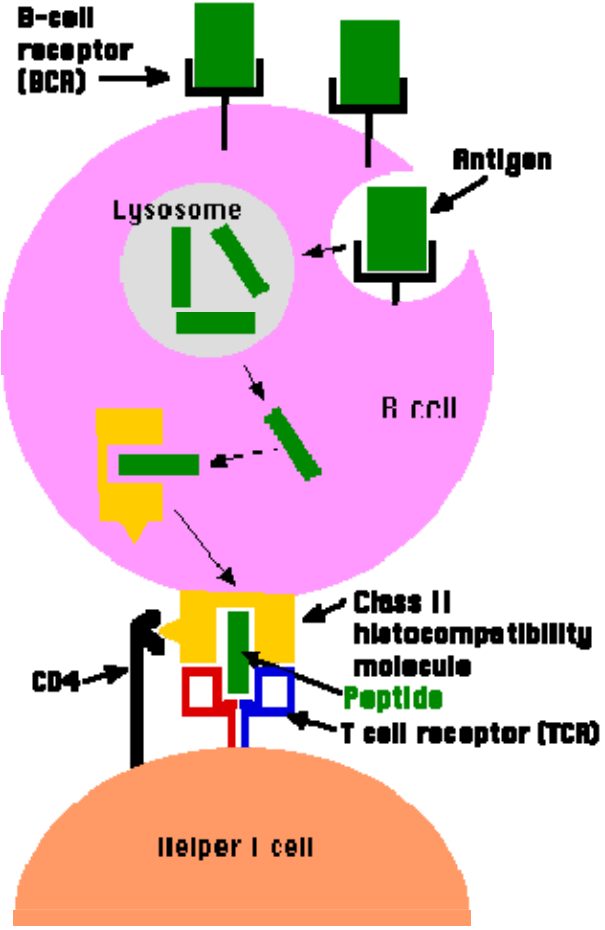
- two transmembrane polypeptides and
- a third molecule nestled in the groove they form.

All three components of this complex must be present in the **RER** for proper assembly.

But antigenic peptides are not transported to the RER, so a protein called the **invariant chain** ("**Ii**") temporarily occupies the groove.

The steps:

- The two chains of the class II molecule are inserted into the membrane of the RER.



- They bind (in their groove) one molecule of invariant chain.
- This trimolecular complex is transported through the Golgi apparatus and into vesicles called lysosomes.

Meanwhile,

- Foreign antigenic material is engulfed by endocytosis forming **endosomes**.
- These also fuse with lysosomes.

Then,

- The antigen is digested into fragments.
- The invariant (Ii) chain is digested.
- This frees the groove for occupancy by the antigenic fragment.
- The vesicles move to the plasma membrane and the complex is displayed at the cell surface.

- The complex can be bound by a **T cell** with
  - a receptor (**TCR**) able to bind the peptide and flanking portions of the histocompatibility molecule (the hot dog in the bun) and
  - **CD4** molecules that bind the **CD4 receptor** (shown above as a yellow triangle) found on all class II histocompatibility molecules.

Link to discussion of how TCRs are synthesized.

External Link
To see animations of these processes, link to immunobiology animations and navigate to "Antigen Recognition" -> "MHC class I processing" and "MHC class II processing" respectively. (Requires Flash 5).
(Please let me know by e-mail if you find a broken link in my pages.)

B Lymphocytes: A Special Case

B lymphocytes process antigen by the class II pathway. However, antigen processing by B cells differs from that of phagocytic cells like macrophages in crucial ways:

- B cells engulf antigen by **receptor-mediated endocytosis**
- The B cell receptors for antigen (**BCR**s) are antibodies anchored in the plasma membrane.

  Link to discussion of how BCRs are synthesized.
- The affinity of these for an epitope on an antigen may be so high that the B cell can bind and internalize the antigen when it is present in body fluids in concentrations thousands of times smaller than a macrophage would need.
- The remaining steps of antigen processing occur by the same class II pathway described above for macrophages producing
- fragments of antigen displayed at the cell surface nestled in the groove of class II histocompatibility molecules.
- A $CD4^+$ T cell that recognizes the displayed antigen is stimulated to release lymphokines.
- These, in turn, stimulate the B cell to enter the cell cycle.
- Because of the part they play in stimulating B cells, these $CD4^+$ T cells are called **Helper T cells** ("**Th**").
- The B cell grows into a clone of cells (called **plasma cells**)
- These synthesize receptors (**BCR**s) with the **identical binding site** for the epitope but without the transmembrane tail.
- These **antibodies** are secreted into the surroundings.

Hemoglobinopathies and Thalassemias

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Diplomate, American Board of Pathology

This is a document in a five-part series on blood cells and anemia:

1. Blood cells and the CBC
2. Anemia: Pathophysiologic Consequences,

5. Hemoglobinopathies and Thalassemias

I. Introduction

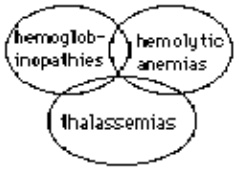
These conditions comprise a very large number of genetic biochemical/ physiological entities, most of which are academic curiosities whose major effect on medicine is to add to the surfeit of useless scientific information. However, several of these conditions (e.g., sickle cell anemia, hemoglobin SC disease, and some thalassemias) are common major life-threatening diseases, and some others (*e.g.*, most thalassemias, hemoglobin E disease, and hemoglobin O disease) are conditions that produce clinically noticeable -- if not serious -- effects and can cause the unaware physician a lot of frustration and the hapless patient a lot of expense and inconvenience. We will study a few hemoglobinopathies and thalassemias of special importance. It should be kept in mind, though, that there are literally hundreds of diseases in these categories.

II. Definitions

Hemoglobinopathy: *A genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule.* Although the suffix "-pathy" would conjure an image of "disease," most of the hemoglobinopathies are not clinically apparent. Others produce asymptomatic abnormal hematologic laboratory findings. A very few produce serious disease. The genetic defect may be due to substitution of one amino acid for another (as with the very common Hb S and Hb C and the great majority of the other abnormal hemoglobins), deletion of a portion of the amino acid sequence (Hb Gun Hill), abnormal hybridization between two chains (Hb Lepore), or abnormal elongation of the globin chain (Hb Constant Spring). The abnormal chain that results may be the α chain (Hb $G_{Philadelphia}$), β chain (Hb S, Hb C), γ chain (Hb F_{Texas}), or δ chain (Hb $A_{2Flatbush}$). These abnormal hemoglobins can have a variety of physiologically significant effects, discussed below in greater depth, but the most severe hemoglobinopathies (Hb S and Hb C diseases) are characterized by hemolysis.

Thalassemia: *A genetic defect that results in production of an abnormally low quantity of a given hemoglobin chain or chains*. The defect may affect the α, β, γ, or δ chain, or may affect some combination of the β, γ, and δ chain in the same patient (but never the α and β chain together). The result is an imbalance in production of globin chains and the production of an inadequate number of red cells. The cells which are produced are hypochromic/microcytic and contain a surfeit of the unaffected chains which cannot stoichiometrically "mate" with the inadequate supply of thalassemic chains. These "bachelor" chains can produce adverse effects on the red cell and lead to destruction of the red cell in the marrow (ineffective erythropoiesis) and in the circulation (hemolysis). Note that

these two definitions are not mutually exclusive -- some hemoglobinopathies may also be thalassemias, in that a structurally abnormal hemoglobin (hemoglobinopathy) may also be underproduced (thalassemia). Some, but not all, hemoglobinopathies and thalassemias are hemolytic anemias. These nosologic concepts are summarized by the Venn diagram below.



III. Pathophysiology of hemoglobinopathies

Messing around with the amino acid sequence of a globin chain has something of a red kryptonite effect. While some positions on the protein chain can tolerate a lot of substitutions without compromising the physiologic integrity of hemoglobin, other positions are very sensitive to amino acid substitutions. For instance, substitution of valine or lysine for glutamate at position 6 of the β chain produces hemoglobins S and C, respectively, which form intraerythrocytic tactoids (see below) and crystals (again respectively) that cause premature destruction of the rbc (hemolysis). On the other hand, substitution of glutamate, asparagine, and threonine for lysine at position 59 of the β chain produces, respectively, hemoglobins $I_{High\ Wycombe}$, J_{Lome}, and $J_{Kaoshiung}$, all of which are physiologically indistinguishable from normal Hb A. Without venturing too deeply into tedious stereochemistry, we can say that abnormal globin structure can functionally manifest itself in one or more of the following ways:

- **Increased O_2 affinity**

  These hemoglobins tend to result when mutations affect the portions of the amino acid sequence that compose 1) the regions of contact between α and β chains, 2) the C-terminal regions, and 3) the regions that form the pocket which binds 2,3-DPG. The hemoglobin eagerly scarfs up the O_2 from the alveoli but then only stingily gives it up to the peripheral tissues. The kidney, always compulsively vigilant for hypoxia, cranks out the erythropoietin thinking that a few extra red cells might help out matters. Erythropoiesis then is stimulated, even though there is no anemia, and erythrocytosis (increased total body rbc mass, increased blood hemoglobin concentration, increased hematocrit) is the result.

  It is important to know that these rare increased O_2 affinity hemoglobins exist to prevent diagnostic errors from occurring in working up patients presenting with erythrocytosis (which is much more commonly caused by other conditions, including polycythemia vera [a neoplasm], cigarette smoking, psychosocial stress, chronic residence at high altitudes, and chronic lung disease). Examples of these include Hb Chesapeake and Hb JCapetown.

- **Decreased O_2 affinity**

  This is the other side of the coin. These hemoglobins are reluctant to pick up O_2 from the lung. The result is a decreased proportion of hemoglobin that is oxygenated at a given PO_2. The remainder of the hemoglobin is, of course, deoxygenated and is blue. If the level of blue hemoglobin exceeds 5 g/dL in capillary blood, the clinical result is cyanosis, a bluish discoloration of skin and mucous membranes.

  Again, it is important to know about these hemoglobins and keep them in the back of your mind when working up cases of cyanosis, a condition much more commonly caused by pulmonary dysfunction or right-to-left cardiovascular shunts. Examples of low O_2 affinity hemoglobins include Hb Seattle, Hb Vancouver, and Hb Mobile.

- **Methemoglobinemia**

  These hemoglobins are a special class of low O_2 affinity hemoglobin variants that are characterized by the presence of heme that contains iron in the ferric (Fe^{+++}) oxidation state, rather than the normal ferrous (Fe^{++}) state. These *methemoglobins* are all designated "Hb M" and further divided by the geographic site of their discovery, e.g., Hb $M_{Saskatoon}$ and Hb $M_{Kankakee}$. The affected patients have **cyanosis**, since the methemoglobin is useless in O_2 binding.

  Methemoglobinemia due to hemoglobinopathy should be distinguished from methemoglobinemia due to other causes, such as **NADH-diaphorase deficiency**. This enzyme is needed for the reduction (to heme) of metheme that accumulates as a result of normal metabolic processes. Congenital absence of NADH-diaphorase causes an accumulation of metheme, despite the fact that the structure of the globin chain is normal. **Toxic methemoglobinemia** occurs in normal individuals exposed to certain oxidizing drugs and other compounds in the environment, even though these individuals have normal hemoglobin structure and a normal complement of NADH-diaphorase. In such victims, the oxidizing power of the toxin overwhelms the normal antioxidant defenses.

  Since methemoglobin is a brown pigment, patients with clinically severe methemoglobinemia have *obviously brown blood*. This observation allows one to make a clever and memorable diagnosis at the bedside during the patient's first venipuncture.

- **Unstable hemoglobin (Heinz body anemia)**

  Certain abnormalities in the globin chain sequence produce a hemoglobin that is intrinsically unstable. When the hemoglobin destabilizes, it forms up into erythrocyte inclusions called Heinz bodies. It is important to know that *Heinz bodies are not visible in cells stained with the routine Wright stain*. It

is necessary for the cells to be stained with a supravital dye (such as brilliant cresyl blue, which can also be used to demonstrate reticulocytes) to be visible. These inclusions attach to the internal aspect of the rbc membrane and reduce the deformability of the cell and basically turn it into spleenfodder. The result is hemolytic anemia. All of these hemoglobins are rare; inheritance is autosomal dominant. Homozygotes have not been described. Examples of unstable hemoglobins are Hb Gun Hill, Hb Leiden, and Hb Köln.

- **Sickling and crystallization**

  These phenomena occur respectively in Hb S and Hb C, the most important of the abnormal hemoglobins. We will deal with these in greater depth next.

IV. Specific hemoglobinopathies

A. Hemoglobin S and sickle cell disease

1. Epidemiology and genetics

The Hb S gene is found primarily in populations of native tropical African origin (which include most African-Americans). The incidence of the gene in some African populations is as high as 40%; in African-Americans the incidence is 8%. The gene is also found with less frequency in non-Indo-European aboriginal peoples of India and in the Middle East. Rare cases have been reported in Caucasians of Mediterranean descent. The gene established itself in the tropical African population presumably because its expression in heterozygotes (**sickle cell trait**) affords some protection against the clinical consequences of *Plasmodium falciparum infestation. Unfortunately, homozygous expression produces **sickle cell disease**, which is a chronic hemolytic anemia and vaso-occlusive condition that usually takes the life of the patient.*

*Hemoglobin S has the peculiar characteristic of expressing its biochemical instability by precipitating out of solution and forming up into long microtubular arrays called **tactoids.** The erythrocytes which contain the Hb S stretch around the tactoids to form the characteristic long, pointed, slightly curved cells called (with somewhat liberal imagination) "sickle cells." Only the deoxygenated form of Hb S (deoxy-Hb S) makes tactoids. The greater the proportion of Hb S in the cell, the greater is the propensity to sickle. Therefore, persons with 100% Hb S (being homozygotes) sickle under everyday conditions, while typical heterozygotes (who usually have about 30-40% Hb S) do not sickle except possibly under extraordinary physiologic conditions. Since Hb S is a β-chain mutation, the disease does not manifest itself until six months of age; prior to that time the Hb S is sufficiently "watered down" by Hb F ($\alpha_2\gamma_2$), which of course has no β-chain.*

In post-infancy individuals homozygous for the Hb S gene, 97+% of the hemoglobin is Hb S, the remainder being the normal minor hemoglobin, Hb A_2 (α

$_2\delta_2$). Several coexisting genetic "abnormalities" (actually godsends) prevalent in African populaitons may ameliorate the course of the disease:

1. ***α-thalassemia** carriers (which comprise 20% of African-Americans!) have a lower MCHC than normal individuals. It has been suggested that a low MCHC is beneficial in decreasing the vaso-occlusive properties of sickled cells. These sickle cell patients live longer and have a milder disease than do non-thalassemic patients. Thalassemia is discussed in greater detail below.*

2. ***Hereditary persistence of fetal hemoglobin** (HPFH) has established itself in the black population and allows Hb F to so dilute the Hb S that sickling does not occur or is less prominent. In these people the Hb F gene does not "turn off" in infancy but persists indefinitely.*

3. ***G-6-PD deficiency** has been suggested as an ameliorative condition for sickle cell disease. This is controversial; the pathophysiologic basis of any such effect must be pretty obscure.*

2. Clinical findings

Sickle cell anemia is a particularly bad disease in that not only is it a hemolytic anemia, but also a vaso-occlusive condition. The clinical findings can then be divided into one of these two groups:

- ***a. Effects of chronic hemolysis***

    1. ***Anemia.** Pretty much self-explanatory*

    2. ***Jaundice**, due to rapid heme turnover and subsequent generation of bilirubin*

    3. ***Cholelithiasis**. It has been classically taught that sickle cell patients are prone to the formation of calcium bilirubinate gallstones due to excess bilirubin secretion into the hepatobiliary tree.*

    4. ***Aplastic crisis**. Many of us have brief episodes of marrow aplasia as a result of common viral infections. With a normal erythrocyte life span of 120 days, no anemia results from an unnoticed marrow shut-down of a few days. However, the sickle cell patients, with their markedly abbreviated rbc life span, can have a precipitous fall in hematocrit (and retic count) under such conditions. This may be life-threatening.*

    5. ***Hemolytic crisis**. Most sickle cell patients establish a stable, tonic level of hemolysis. Rarely, for obscure reasons, they experience a catastrophic fall in hematocrit, increasing intensity of jaundice, and increasing reticulocyte count. This is called a "hemolytic crisis."*

- ***b. Effects of vaso-occlusion***

1. ***Dactylitis**. Resulting presumably from infarction or ischemia of the bones of the hands and feet, this is often the presenting manifestation of sickle cell disease in a six-months-old infant. The hands and feet are swollen and painful.*

2. ***Autosplenectomy**. In childhood, the spleen is enlarged due to excess activity in destruction of the sickled erythrocytes. Gradually, the spleen infarcts itself down to a fibrous nubbin.*

3. ***Priapism**. This refers to a painful and sustained penile erection, apparently due to sludging of sickled cells in the corpora cavernosa. Sometimes the penis has to be surgically decompressed. Repeated episodes of priapism cause the spongy erectile tissues to be replaced by fibrous tissue, with impotence being the end result.*

4. ***Renal papillary necrosis**. The physiologic function of the loops of Henle make the renal medulla an eldritch, unbodylike area of high hematocrit, high osmolarity, low pH, hemodynamic stasis, and low P_{O2}. All of these conditions predispose to sickling and infarctive loss of the papillae of the pyramids. The result is inability to concentrate and dilute urine. Even sickle cell trait individuals may experience episodes of hematuria, presumably due to this mechanism.*

5. ***Infarctive (painful) crisis**. Increased sickling activity may be brought about by any general stress on the body, especially infection. Almost any organ may suffer acute infarction (includinmg the heart), and pain is the chief symptom.*

6. ***Sequestration crisis**. This occurs mostly in infants and young children and is characterized by sudden pooling of sickled erythrocytes in the RES and vascular compartment. This produces a sudden fall in hematocrit. Sequestration crisis may be the most common cause of death in sickle cell patients in the youngest age group.*

7. ***Leg ulcers**. After all of the disasters mentioned above, this seems trivial. However, the deep, nonhealing ulcers of skin and tela subcutanea (classically around the medial malleolus) may be the only clinical manifestation of sickle cell disease in an otherwise well-compensated patient. These may be the only bugaboo standing between the patient and a productive, financially solvent life.*

B. Hemoglobin C

*The gene for Hb C is also prevalent in the African-American population but with less frequency (2-3%) than that of the sickle cell gene. Hb C does not form tactoids, but intracellular blunt ended **crystalloids**. The result is decreased rbc*

survival time; however, hemolysis is not as severe as in sickle cell disease, and the vaso-occlusive phenomena, so devastating in sickle cell disease, are not generally noted. Like sickle cell trait, the Hb C trait is asymptomatic. Homozygotes (and some heterozygotes) for Hb C often have many target cells (codocytes) in the peripheral smear, but the crystals, although pathognomonic, are only occasionally seen. The prognosis of homozygous Hb C disease is excellent.

An individual may inherit a Hb S gene from one parent and a Hb C gene from the other. The result of this double whammy is ***Hb SC disease****. The clinical severity of this condition is intermediate between that of sickle cell disease and Hb C disease, except that visual damage due to retinal vascular lesions is characteristically worse in SC disease than in sickle cell anemia. The intracellular bodies that occur upon hemoglobin destabilization in SC disease are curious hybrids of the blunt-ended crystalloids of Hb C and the sharp-pointed tactoids of Hb S, in that they often have one pointed end and one blunt end, thus vaguely resembling arrowheads.*

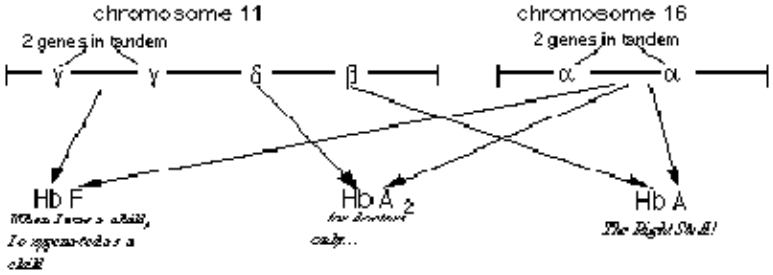
C. Hemoglobin E

This is a very common β-chain mutation among Southeast Asians. The Thai and Khmer groups have the highest frequency, followed by Burmese and Malays, then Vietnamese and Bengalis. The gene does not occur in ethnic Han Chinese or Japanese. The heterozygous state is asymptomatic but causes microcytosis without anemia, thus resembling some cases of β-thalassemia minor (see below). The homozygous state has more severe microcytosis and hypochromia, but little, if any, anemia (this is also reminiscent of thalassemia minor). Hemoglobin E should always be considered working up an unexplained microcytosis in a member of one of the affected ethnic groups.

V. Thalassemia

A. Genetics

Understanding the thalassemias can be facilitated by reviewing the genesis of the normal post-embryonal hemoglobins:



Chromosome 16 contains the genes for the all-important α-chain. The genes for all of the other important globin chains are on chromosome 11, where they are closely linked. The linkage means (if you will briefly abuse yourself by recalling

basic genetics) that the genes tend to be inherited as a group, as opposed to non-linked (or distantly linked) genes which assort independently due to crossing over during gametogenesis. Because of the linkage, a mutation that affects the rate of production of the β-chain not uncommonly affects rate of production of the adjacent δ-chain. An individual carrying such a mutation would then have a gene for "δβ thalassemia." He or she could pass on the δβ-thalassemia gene to offspring but would essentially never, say, pass a δ-thalassemia gene to one child and a β-thalassemia gene to another. Conversely, since the genome for the α-chain is on a completely different chromosome than the genes for all the other chains, one would expect no mutation in a chromosome 11 chain gene (δ,β,γ) to affect α-chain production. Moreover, if some poor shlimazel happened to inherit an α-thalassemia gene from one parent and a β-thalassemia gene from another, he would not tend to pass both abnormal genes on as a unit to his or her offspring. One kid (out of a representative Mendelian sibship of four) would get the abnormal α-gene, one would get the abnormal β, one would get neither, and one would get both.

B. Biochemistry and pathophysiology

But enough of Mendel! We're in med school to learn about hemoglobin, right? Whatever the genetics, the clinical problem in the thalassemias is the inability to maintain a balance between the synthesis rate of one type of globin chain vis-à-vis that of its mate. Even though thalassemias have been described for all four of the above chains, we will consider only those that involve the β-chain (the β-thalassemias and δβ-thalassemias) and the α-chain (α-thalassemias). It will be useful to review what kind of hemoglobins you can build by mixing and matching globin chains:

Hemo-globin	Globin chain composition	Notes
A	$\alpha_2\beta_2$	The only physiologically important adult hemoglobin in normal individuals. Includes the post-translational glycosylated hemoglobins A1a, A1b, and A1c, the last being important in monitoring diabetics.
F	$\alpha_2\gamma_2$	The major physiologic hemoglobin in post embryonal fetuses. Adapted best for lowered intrauterine O_2 tension because of its left-shifted Hb-O_2 dissociation curve (allowing O_2 to be more readily picked up from maternal circulation). Production normally turns off in early infancy. Proportion of circulating Hb F fades to insignificance at about 6 months of age.
A_2	$\alpha_2\delta_2$	Medical philosopher's proof of the existence of God (and God's love of physicians). Apparently put here solely as a marker for doctors trying to figure out whether a patient has iron deficiency anemia or β-thalassemia. Normally

		less than 3% of circulating hemoglobin (thus physiologically insignificant), Hb $A_2$is slightly elevated in most βthalassemias, but normal or decreased in iron deficiency, thus making it a nifty marker for evaluating microcytic, hypochromic anemias.
Gower 1 **Gower 2** **Portland**	$\zeta_2\varepsilon_2$ $\alpha_2\varepsilon_2$ $\zeta_2\gamma_2$	Very early normal embryonal hemoglobins that disappear after 8 weeks of gestation. The only one of clinical importance is Hb Portland, which may be seen at birth in cases of the severest form of αthalassemia.
H	β_4	Abnormal hemoglobin produced in cases of α thalassemia, when excess βchains decide to get it on with each other, there not being enough αchains to go around. Intrinsically unstable, Hb H produces Heinz bodies in the erythrocytes and subsequent hemolysis.
Bart's	γ_4	Delinquent youth gang analogue of Hb H. This abnormal hemoglobin is found in *infants* with αthalassemia. Detecting presence of Hb Bart's in cord blood may be the only practical way to screen for the very large number of individuals who are silent carriers of one type of αthalassemia (see below).

C. Beta thalassemia

Although this is the classic form of thalassemia it is not the most common. The first description was written by Dr. Thomas Cooley in 1925. The term "Cooley's anemia" has been used synonymously with clinically severe forms of β thalassemia, although the remainder of Cooley's career was so undistinguished as to cause some to suggest that his name is not worthy of eponymous immortality. Cooley's anemia was a fatal microcytic anemia of children of Mediterranean descent. The name "thalassemia" was coined to reflect the original geographic home of the target population ("thalassa" is the classical Greek name for the Mediterranean Sea). Over the years, it became clear that many other groups (Africans, African-Americans, Arabs, Indians, and Southeast Asians) are affected. In fact, thalassemias in general tend to affect races of people that hail from a tropical belt that girdles the Mediterranean and extends all the way through the Indian subcontinent to Southeast Asia.

There are a multiplicity of different βthalassemia genes that give rise to a clinically heterogeneous spectrum ranging from asymptomatic expression to classical, deadly Cooley's anemia. It is convenient to group the various βthalassemias into two groups, based on the amount of βglobin chain production:

- ***β^0 thalassemia***

  This abnormal gene allows no production of βchains. Individuals homozygous for this gene produce only Hb A_2, Hb F (and very little of that after six months of age), and unstable α_4 ***tetramers*** *that trash the red cells*

while they are still in the marrow. As you might imagine, these people are in pretty dire straits unless some guardian angel has given them another, independent gene for hereditary persistence of fetal hemoglobin (HPFH). This prevents the Hb F spigot from turning down to a trickle at six months. Such persons can live to ripe old age and still be young at heart.

- **β^+ *thalassemia***

  *This abnormal gene allows some, but still subnormal, production of β chains. People homozygous for this gene will make a subnormal amount of Hb A but will still have trouble with the destructive effects of α_4 tetramers on the erythrocytes and erythrocyte precursors in the marrow. The β^+ genes can be further subdivided into the classic β^+ **(severe)** form, seen in Mediterranean Caucasians, and the mild β^+ **(Negro)** form seen in blacks. Nowadays this gene has its highest population concentration in Liberia.*

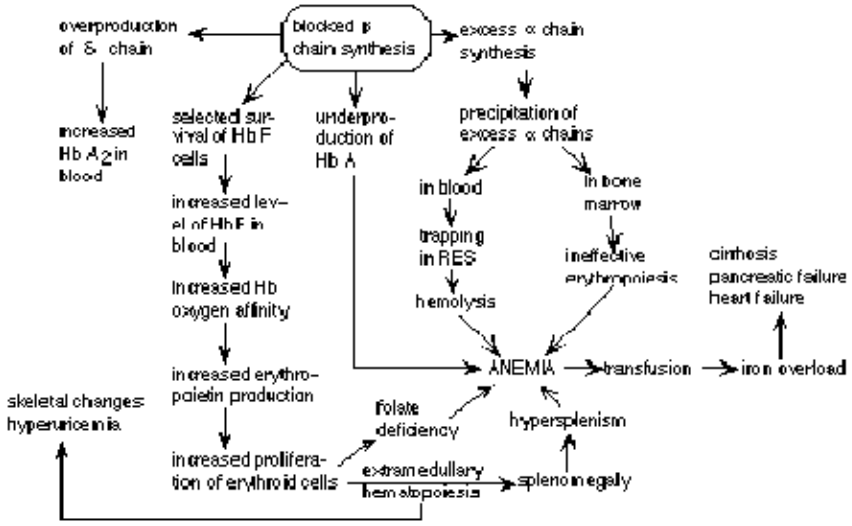
Although these genes are remarkably varied in their effect on βchain synthesis rate, one can make up some useful rules of thumb:

1. *Individuals heterozygous for any of the βthalassemia genes are either silent carriers or have minimal clinical effects, usually manifested as a **borderline anemia** (Hct ~ 35 cL/L) with disproportionate microcytosis (MCV ~ 60 fL) and a reciprocally **high rbc count** (~ 6 x 10^6/µL). The Hb A_2 is increased. This clinical presentation is called **thalassemia minor**. It makes for interesting wine-tasting party conversation if you have this condition, and all that your friends can muster is chronic fatigue syndrome. Your kids should have no problems if you just marry a Teuton, Slav, Balt, or Lapp.*

2. *Individuals homozygous for all of the βthalassemia genes [except the β^+ (Negro) gene] have severe anemia and some or all of the pathophysiological consequences given in the diagram below. This is classic Cooley's anemia and is termed **thalassemia major**. This is bad news.*

3. *Individuals homozygous for the β^+ (Negro) gene and several other miscellaneous types of mildly behaving genes have a relatively mild clinical anemia called **thalassemia intermedia.** These patients may require transfusion, but only later in life than is the case in the very sick children with thalassemia major.*

The pathophysiology of βthalassemia major is best understood by going and getting yourself a beer (or politically correct beverage), watching a little TV, doing one or two other chores to postpone the inevitable, and then sitting down to study the next diagram.



While studying the illustration, consider the following observations concerning β thalassemia:

1. *Since there is a decrease in the synthesis of β chains, there is a net decreased synthesis of Hb A. With less Hb A available to fill the red cells, the result is* ***microcytic anemia****. Whereas in iron deficiency microcytosis occurs because there is not enough heme, in thalassemia the same thing occurs because there is not enough globin.*

2. *Since the body cannot make enough β chains, it makes a feeble attempt to compensate by trying to churn out δ chains. The result is* ***increased Hb*** A_2*, which can be measured easily and inexpensively by column chromatography. This is a pretty specific test for the diagnosis of β thalassemia. Pitfall: both β and δ chains are decreased in* ***δβ thalassemia****, which is not rare and presents like β thalassemia, except that the Hb* A_2 *is not elevated. You would expect this since Hb* A_2 *contains δ chains).*

3. *In some cases of β thalassemia, there is attempt at compensation by maintaining some production of* ***Hb F****. This has some pathophysiologic consequences (as shown above) and also provides a laboratory marker to assist in diagnosis. Retention of Hb F production is not as common as increased rbc Hb* A_2 *content.*

4. *In severe forms of thalassemia, the anemia due to failure to make adequate amounts of Hb A is compounded by the hemolysis, ineffective erythropoiesis, and extramedullary hematopoiesis brought on by precipitation of* α_4 *tetramers (which are unstable). In classic Cooley's anemia, the ineffective erythropoiesis dominates the clinical picture by producing tremendous expansion of the marrow space, manifested by the so-called "tower skull" with an x-ray showing innumerable vertical bony striae between the inner and outer tables of the calvarium. This radiographic feature is fancifully called the "hair-on-end appearance" by*

*radiologists, and the "guy-who-accidentally-sat-on-a-Van-de-Graaff-generator appearance" by those wacky electrical engineers. Extramedullary hematopoiesis and hemolysis causes **splenomegaly**, which produces **hypersplenism**, and more hemolysis.*

5. *The high turnover state caused by the tremendous erythroproliferative activity causes wastage of folate and may produce a complicating **megaloblastic anemia**. Another effect of the high turnover state is **hyperuricemia** (due to catabolism of the purine content of cellular DNA).*

6. *Classically in thalassemia major, the treatment is the cause of death. The children are maintained by transfusions until about age ten years, at which time they start to show symptoms of excess iron loading. This happens because the transfusion bypasses the body's normal gastrointestinal mechanism of iron intake and excretion. The iron is poured into the bloodstream directly; the body cannot excrete it fast enough. First iron (as hemosiderin) fills the cytoplasm of the RES phagocytes and then starts to be deposited in the parenchymal cells of just about every organ of the body. The pancreas, liver, myocardium, adrenals, and gonads are among the organs most sensitive to iron toxicity. The clincial result is **diabetes mellitus, hepatic cirrhosis, congestive heart failure, adrenal insufficiency**, and **failure to undergo puberty**. Death used to occur in the second or third decade of life, the most common immediate cause being complications of heart failure. Nowadays, thal major patients live longer because of advances in chelation therapy. To achieve such longevity, they must submit to daily subcutaneous injections of the parenteral chelating agent, deferoxamine. These injections are given by pump, usually overnight, and last 9 to 12 hours each. An oral chelating drug in presently under development in Europe.*

D. Alpha thalassemia

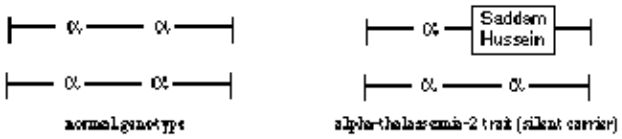
The α-thalassemias include the most common of all hemoglobinopathies and thalassemias. One form of α-thalassemia is very common in African-Americans. Fortunately this form is so mild that its very detection is almost impossible in adult heterozygotes, and even homozygotes are asymptomatic with mild laboratory abnormalities. Yet another form of α-thalassemia, fortunately uncommon, produces the most severe disease of all the hemoglobinopathies and thalassemias and usually takes the life of its victim even before birth. Surely α-thalassemia is a disease of extremes!

It is helpful to consider two concepts concerning α-thalassemia:

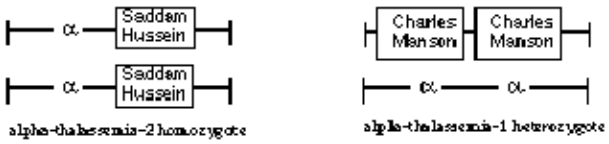
- *Unlike β-thalassemia, α-thalassemia is present even before birth, since the α-chain is integral to all hemoglobins past the very primordial Hb Gower 1 and Hb Portland. Thus, individuals, born and unborn, who carry one of these genes underproduce Hb Gower 2, Hb F, Hb A, and Hb A_2.*

Obviously then, elevated Hb A_2 cannot be used as a diagnostic marker for α-thalassemia.

- *As noted in an illustration above, each individual has four genes for the α globin chain, which can be denoted as αα/αα. Each haplotype (αα) is inherited from a parent as an indestructible, non-crossoverable pair. In α-thalassemias, anywhere from one to all four of these genes of the diplotype (αα/αα) can be deleted (or transformed into some "bad guy" gene that doesn't do anything), producing a dose-related depression of α-chain synthesis. Thus, the (α-/ααα) diplotype produces the mildest condition, while (--/--) produces the fatal intrauterine disease. The (α-) gene is called the "α-thalassemia-2" gene while the (--) gene is termed the "α-thalassemia-1" gene. One can be homozygous or heterozygous for each of these, to give four possible diplotypes (α-/ααα, α-/α-, --/ααα, --/--). An additional diplotype is the double heterozygote for α-thalassemia-1 and α-thalassemia-2 (α-/--). Since the α-thalassemia-1 and α-thalassemia-2 genes tend to occur in different races (Asians and Africans, respectively, between which there is a low rate of intermarriage), this combination is not common. Let us look at the various nefarious gene combinations individually:*

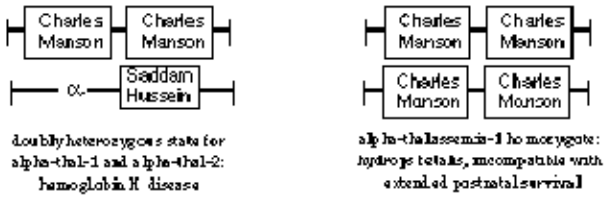


In the above diagram, the normal diplotype is compared with the heterozygous state for α-thalassemia-2. The latter occurs in 20% of all African-Americans. It produces no symptoms and no abnormalities on routine laboratory tests. The only practical way to detect it is to screen all black infants at birth for Hb Bart's (γ_4), and even this technique will miss some individuals. It is thought that the reason for the high prevalence of this gene in the black population is that it may be an "anti-sickle cell" gene. It turns out that Hb S homozygotes have a milder course of sickle cell anemia if they also are silent carriers for α-thalassemia.



The the two diplotypes given above, two of the four α-genes are trashed by "bad guy" mutations. Such individuals are usually not anemic but may have microcytosis. They become victims of the medical system when they are subjected to expensive and time consuming testing in a quixotic search for some serious hematologic condition that does not exist. About the only way to make a diagnosis of one of these conditions is to eliminate other causes of microcytosis

and/or check rbc indices on all available blood (no pun intended) relatives. Amidst all this shenanigans lurks a somber note -- if an α-thalassemia-1 heterozygote finds his or her true love in another α-thalassemia-1 heterozygote, one-fourth of the issue of such a union will suffer the always lethal homozygous state.



*The double heterozygote on the left has "**hemoglobin H disease**," so named because of the presence of a significant proportion of the hemoglobin composed of four β-chains. Affected infants will, of course, show some Hb Bart's as well. These people have a hemolytic anemia which varies from very mild to that which clinically resembles β-thalassemia major.*

*The α-thalassemia-1 homozygote, on the right, is allowed no production of α-chains. The only hemoglobins present are Hb Bart's, Hb H, and Hb Portland. Most of the affected die in utero or within hours after birth. Autopsy shows massive extramedullary hematopoiesis in virtually every parenchymatous organ of the body. The severe anemia causes congestive heart failure and subsequent massive total body edema, termed "**hydrops fetalis**." Parenthetically, it should be noted that hydrops (from which springs the term "dropsy") is not limited to thalassemia but is seen in any conditon that causes severe heart failure in utero, such as in the anemia due to alloimmune hemolytic disease of the newborn.*

[To Ed Uthman's Home Page]

Introduction

Hematopathology is not only the study of disease of the blood and bone marrow, but also of the organs and tissues which employ blood cells as principal effectors of their physiologic functions. Such would include the lymph nodes, spleen, thymus, and the many foci of lymphoid tissue found along the

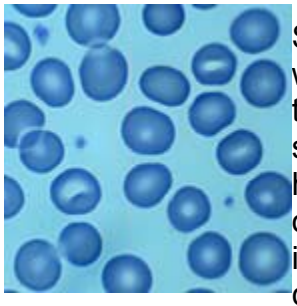
aerodigestive tract. Generally two types of medical subspecialists intensively practice in this area, the **hematologist** and the **hematopathologist**. The hematologist usually is a Board-certified internist who has completed additional years of training in hematology, usually as part of a combined fellowship in hematology and oncology. The thrust of this individual's work is toward the diagnosis and medical management of patients with hematologic disease, especially neoplasms, and medical management of other nonhematologic cancer. The hematopathologist, on the other hand, is usually Board-certified in anatomic and clinical pathology and has taken additional years of training in hematopathology. His or her principal activity is the morphologic diagnosis of conditions of the hematopoietic and lymphocyte-rich tissues and in the performance of laboratory testing that assists such diagnosis.

Hematopathology is somewhat unique in its approach to the patient and the disease, in that 1) many diseases are understood at the molecular level, 2) the patient's tissue is easily obtainable in large quantities (in the case of peripheral blood, at least) and easily kept viable for special studies, and 3) the function of the blood (or at least the erythroid component) is relatively simple when compared to that of other organ systems. Because it is a scientifically integrated discipline hematology/hematopathology is an area which is intellectually gratifying to the eclectic individual who is well-rounded in various biomedical endeavors, including biochemistry, physiology, pharmacology, microanatomy, morphologic diagnosis, and patient care.

The Blood

A few nights working in a trauma center would tend to convince one that the body is just a huge bag of blood. In fact, an "average" 70 liter human body contains only about 5 liters of blood, or 7% by volume. In the normal state, blood has no business anywhere except in the confines of the heart and blood vessels and in the sinusoids of the marrow, liver, and spleen. Of the average 5 L of blood, only 2.25 L, or 45%, consists of cells. The rest is plasma, which itself consists of 93% water (by weight) and 7% solids (mostly proteins, the greatest proportion of which is albumin). Of the 2.25 L of cells, only 0.037 L (1.6%) are leukocytes. The entire circulating leukocyte population, if purified, would fit in a bartender's jigger. The total circulating platelet volume is even less -- about 0.0065 L -- or a little over one teaspoonful.

Erythrocytes



Structurally the simplest cell in the body, volumes have been written about the lowly red blood cell. The basic function of the rbc is the creation and maintenance of an environment salutary to the physical integrity and functionality of hemoglobin. In the normal state, erythrocytes are produced only in the skeleton (in adults only in the axial skeleton), but in pathologic states (especially myelofibrosis, which will be covered subsequently) almost any organ can become the

site of erythropoiesis. Numerous substances are necessary for creation of erythrocytes, including metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamin), and amino acids. Regulatory substances necessary for normal erythropoiesis include erythropoietin, thyroid hormones, and androgens. Erythrocytes progress from blast precursors in the marrow over a period of five days. Then they are released into the blood as reticulocytes, distinguishable from regular erythrocytes only with special supravital stains. The reticulocyte changes to an erythrocyte in one day and circulates for 120 days before being destroyed in the reticuloendothelial system.

Clinical laboratories measure several important parameters that reflect rbc structure and function. These measurements are used to 1) evaluate the adequacy of oxygen delivery to the tissues, at least as is related to hematologic (as opposed to cardiopulmonary) factors, and 2) detect abnormalities in rbc size and shape that may provide clues to the diagnosis of a variety of hematologic conditions. Most of these tests are performed using automated equipment to analyze a simple venipuncture sample collected in a universal lavender- (or purple-) top tube containing EDTA as an anticoagulant. Let us consider each of these tests.

- **A. Hemoglobin concentration in whole blood**

  Referred to simply as "hemoglobin," this test involves lysing the erythrocytes, thus producing an evenly distributed solution of hemoglobin in the sample. The hemoglobin is chemically converted mole-for-mole to the more stable and easily measured cyanmethemoglobin, which is a colored compound that can be measured colorimetrically, its concentration being calculated from its amount of light absorption using Beer's Law. The normal range for hemoglobin is highly age- and sex-dependent, with men having higher values than women, and adults having higher values than children (except neonates, which have the highest values of all). For a typical clinical lab, the young adult female normal range is 12 - 16 g/dL; for adult males it is 14 - 18 g/dL.

  This is an easy test to perform, as hemoglobin is present in the blood in higher concentration than that of any other measured substance in laboratory medicine. The result is traditionally expressed as unit mass per volume, specifically grams per deciliter (g/dL). Ideologues in lab medicine have been maintaining for years that this unit will be replaced by Système Internationale (SI) units of moles per liter, but this has not gained any significant acceptance in clinical medicine except in the most nerdly circles.

- **B. Erythrocyte count**

  Also referred to as just "rbc," this simply involves counting the number of rbcs per unit volume of whole blood. Manual methods using the hated hemocytometer have been universally replaced by automated counting.

The major source of error in the rbc count is an artificially reduced result that occurs in some conditions where rbcs stick together in the sample tube, with two or more cells being counted as one. The result of the test is expressed as number of cells per unit volume, specifically cells/μL. A typical lab's normal range is 4.2 - 5.4 x 10^6/μL for females; for adult males it is 4.7 - 6.1 x 10^6 /μL.

- **C. Hematocrit**

  This is also called the packed cell volume or PCV. It is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. The result is expressed as a proportion, either unitless (e.g., 0.42) or with volume units (e.g., 0.42 L/L, or 42 cL/L [centiliters/liter]). An archaic way of expressing hematocrit is "volumes per cent" or just "percent" (42%, in the above illustration). Small office labs and stat labs measure hematocrit simply by spinning down a whole blood sample in a capillary tube and measuring the length of the column of rbcs relative to the length of the column of the whole specimen. Larger labs use automated methods that actually measure the volume individually of each of thousands of red cells in a measured volume of whole blood and add them up. The volume of individual erythrocytes can be electronically determined by measurement of their electrical impedance or their light-scattering properties. The normal range is 0.37 - 0.47 L/L for females, and 0.42 - 0.52 L/L for males.

- **D. Erythrocyte indices**

  The three cardinal rbc measurements described above (hemoglobin, hematocrit, and rbc count) are used to arithmetically derive the erythrocyte indices - mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. As much as we all hate memorization, it is important to know how to calculate these indices and have some idea of the normal ranges. We will consider these individually.

  - **1. Mean corpuscular volume (MCV)**

    This is the mean volume of all the erythrocytes counted in the sample. The value is expressed in volume units, in this case very small ones - femtoliters (fL, 10-15 liter). The normal range is 80 - 94 fL. The formula for the calculation in general terms is

    MCV = hematocrit ÷ rbc count

    When using specific units, decimal fudge factors are required; for example,

    MCV (in fL) = (hematocrit [in L/L] x 1000) ÷ (rbc count [in millions/μL])

    I think that it is easier to forget the fudge factors, use the first formula, multiply out the values while ignoring the bothersome

decimal, and reposition the decimal in the final result so as to approximate the order of magnitude of the normal range. This is safe, since you will not see an MCV of 8 fL, or one of 800 fL.

When the MCV is low, the blood is said to be (strong>microcytic</STRONG**macrocytic**. **Normocytic** refers to blood with a normal MCV. Keep in mind that the MCV measures only average cell volume. The MCV can be normal while the individual red cells of the population vary wildly in volume from one to the next. Such an abnormal variation in cell volume is called **anisocytosis**. Some machines can measure the degree of anisocytosis by use of a parameter called the **red cell distribution width (RDW)**. This is simply a standardized parameter (similar to the standard deviation) for mathematically expressing magnitude of dispersion of a population about a mean. The normal range for RDW is 11.5 - 14.5 %.

- **2. Mean corpuscular hemoglobin (MCH)**

  The MCH represents the mean mass of hemoglobin in the RBC and is expressed in the mass unit, picograms (pg, 10^{-12} gram). The value is determined by the formula,

  MCH (in pg) = (hemoglobin [in g/dL] x 10 ÷ (rbc count [in millions/μL])

  Again, a fudge factor is required in this equation, so it helps to get some feel for the normal range (27 - 31 pg) and gestalt the decimal point, as described for MCV, above. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV. The MCH is something of a minor leaguer among the indices in that it adds little information independent of the MCV.

- **3. Mean corpuscular hemoglobin concentration (MCHC)**

  This is the mean concentration of hemoglobin in the red cell. Since whole blood is about one-half cells by volume, and all of the hemoglobin is confined to the cells, you would correctly expect the MCHC to be roughly twice the value for hemoglobin in whole blood and to be expressed in the same units; the normal range is 32 - 36 g/dL. The value is calculated using the formula,

  MCHC [in g/dL] = hemoglobin [in g/dL] ÷ hematocrit [in L/L]

  Cells with normal, high, and low MCHC are referred to as **normochromic, hyperchromic, and hypochromic**, respectively. Again, these terms will have importance in anemia classification.

Further reading on red cell disease

Anemia: Pathophysiologic Consequences, Classification, and Clinical Investigation is an introduction to anemia

Nutritional Anemias and Anemia of Chronic Disease deals with anemias caused by iron, folate, and vitamin B_{12} deficiencies.

Hemolytic Anemias is concerned with anemias caused by red cells being destroyed faster than a healthy marrow can replace them.

Hemoglobinopathies and Thalassemias covers sickle cell disease, hemoglobins C and E, and alpha- and beta-thalassemias.

Understanding Anemia, my first book, is now available in hardback and paper. The publisher has kindly allowed me to post the full text of Chapter 1 online. You can access it through the book outline at this link. There is also a link to buy the book from online bookstores at a substantial discount. This book is aimed at general readers and presumes a knowledge of biology at the high school level, then builds from there.

Leukocytes and the leukocyte differential count

To consider the leukocytes together as a group is something of a granfalloon, because each type of leukocyte has its own function and ontogeny semi-independent of the others. To measure the total leukocyte count and allow this term to mean anything to the doctor is a travesty, yet the "wbc" count has traditionally been considered a cardinal measurement in a routine laboratory workup for just about any condition. I cannot emphasize too much that to evaluate critically the hematologic status of a patient, one must consider the individual absolute counts of each of the leukocyte types rather than the total wbc count. For such a critical evaluation, the first step is to order a wbc count with differential. In many labs, the result will be reported as a relative differential, something like this:

WBC	6000/μL
segmented neutrophils	60%
band neutrophils	2%
lymphocytes	25%
monocytes	8%
eosinophils	3%
basophils	2%

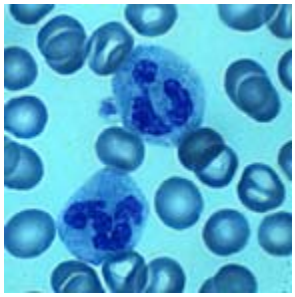
Your first task is to multiply the wbc count by each of the percentages given for the cell types; this gives you an **absolute differential**. Now you're in business to get some idea as to the pathophysiologic status of the patient's blood and marrow. Thus, the illustration above becomes:

WBC	6000/μL

segmented neutrophils	3600/μL
band neutrophils	120/μL
lymphocytes	1500/μL
monocytes	480/μL
eosinophils	180/μL
basophils	120/μL

The total wbc count is invariably done using an automated method. Routinely, the differential count is done "by hand" (i.e., through the microscope) in smaller labs, and by automated methods in larger facilities. The automated methods are amazingly accurate, considering the fine distinctions that must often be made in discerning one type of leukocyte from the other. One manufacturer's machine can quite reliably pick out one leukemic blast cell in eight hundred or more leukocytes. Now we shall consider each of the leukocyte types individually.

- **A. Neutrophils**



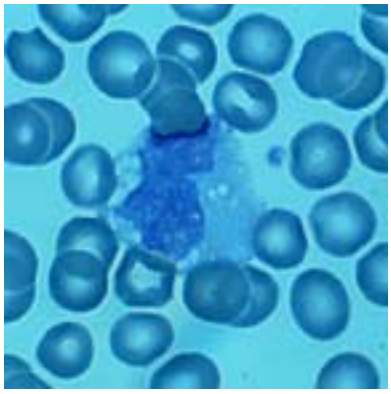
The most populous of the circulating white cells, they are also the most short lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, infarction, trauma, emotional distress, or other noxious stimuli. When called to a site of injury, they phagocytose invaders and other undesirable substances and usually kill themselves in the act of doing in the bad guys.

Normally, the circulating neutrophil series consists only of band neutrophils and segmented neutrophils, the latter being the most mature type. In stress situations (i.e., the "acute phase reaction"), earlier forms (usually no earlier than myelocytes) can be seen in the blood. This picture is called a "left shift." The band count has been used as an indicator of acute stress. In practice, band counts tend to be less than reliable due to tremendous interobserver variability, even among seasoned medical technologists, in discriminating bands from segs by microscopy. Other morphologic clues to acute stress may be more helpful: in the acute phase reaction, any of the neutrophil forms may develop deep blue cytoplasmic granules, vacuoles, and vague blue cytoplasmic inclusions called Döhle bodies, which consist of aggregates of ribosomes and endoplasmic

reticulum. All of these features are easily seen (except possibly the Döhle bodies), even by neophytes.

The normal range for neutrophil (band + seg) count is 1160 - 8300 /µL for blacks, and 1700 - 8100 /µL for other groups. Keeping in mind the lower expected low-end value for blacks will save you much time (and patients much expense and pain) over the course of your career. Obesity and cigarette smoking are associated an increased neutrophil count. It is said that for each pack per day of cigarettes smoked, the granulocyte count may be expected to rise by 1000 /µL.

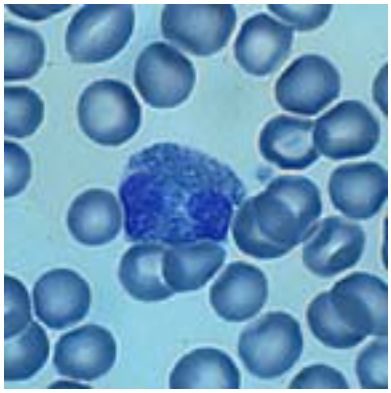
- **B. Monocytes**



These large cells are actually more closely related to neutrophils than are the other "granulocytes," the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. Monocytes are to histiocytes (or macrophages) what Bruce Wayne is to Batman. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Here they serve as the welcome wagon for any outside invaders and are capable of "processing" foreign antigens and "presenting" them to the immunocompetent lymphocytes. They are also capable of the more brutal activity of phagocytosis. Unlike neutrophils, histiocytes can usually survive the phagocytosis of microbes. What they trade off is killing power. For instance, mycobacteria can live in histiocytes (following phagocytosis) for years.

The normal range for the monocyte count is 200 - 950 /µL.

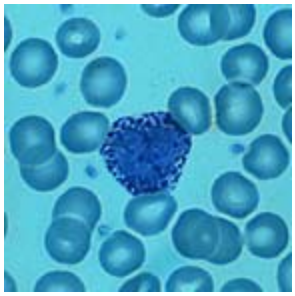
- **C. Eosinophils**



These comely cells are traditionally grouped with the neutrophils and basophils as "granulocytes," another granfalloon. Current thinking is that eosinophils and neutrophils are derived from different stem cells, which are not distinguishable from each other by currently available techniques of examination. Although the hallmark of the eosinophil is the presence of bright orange, large, refractile granules, another feature helpful in identifying them (especially on H&E-stained routine histologic sections) is that they rarely have more than two nuclear lobes (unlike the neutrophil, which usually has three or four). The normal range of the absolute eosinophil count is 0 - 450 /µL.

Eosinophils are capable of ameboid motion (in response to chemotactic substances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The eos may serve a critical function in mitigating allergic responses, since they can 1) inactivate slow reacting substance of anaphylaxis (SRS-A), 2) neutralize histamine, and 3) inhibit mast cell degranulation. The life span of eos in the peripheral blood is about the same as that of neutrophils. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises.

- **D. Basophils**



The most aesthetically pleasing of all the leukocytes, the basophils are also the least numerous, the normal range of their count in peripheral blood being 0 - 200/µL. They are easily recognized by their very large, deep purple cytoplasmic granules which overlie, as well as flank, the nucleus (eosinophil granules, by contrast, only flank the nucleus but do not overlie it). It is tempting to assume that the

basophil and the mast cell are the blood and tissue versions, respectively, of the same cell type. Actually it is controversial as to whether this concept is true or whether these are two different cell types.

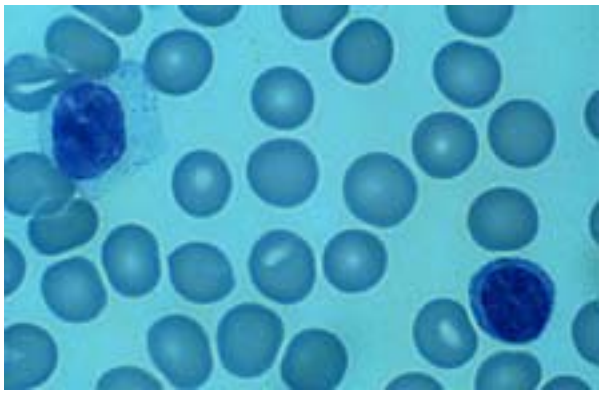
- The table below presents some of the contrasts between mast cells and basophils.

Feature	Basophils	Mast cells
Nuclear morphology	segmented	round or ovoid
Mitotic potential	no	yes
Peroxidase content	+	-
Acid phosphatase	-	+
Alkaline phosphatase	-	+
PAS reaction	++++	+

- In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase. This reciprocal relationship suggests that they represent the same cell type (i.e., an allergen stimulates the passage of the cells from the blood to the site of the allergen in the tissues). Some experiments with animals have also shown that mast cells are marrow-derived and are capable of differentiating into cells that resemble basophils. Conversely, some recent evidence suggests that basophils (as well as eosinophils) can differentiate from metachromatic precursor cells that reside among epithelial cells in the nasal mucosa

- Without invoking religion or Alexander Pope ("Whatever is, is right," *An Essay on Man*, 1732-34) it is hard to see any useful role of the basophil/mast cell in human physiology. The mast cell is the essential effector of immediate (Type 1) hypersensitivity reactions, which produce only misery, dysfunction, and occasionally death for the hapless host.

- **E. Lymphocytes**



In the immune/inflammatory response, if the neutrophils and monocytes are the brutes, the lymphocytes are the brains. It is possible to observe the horror of life without lymphocyte function by studying the unfortunate few with hereditary, X-linked, severe combined immune deficiency. Such individuals uniformly die of systemic infections at an early

age (except for the "bubble boys" of yesteryear, who lived out their short lives in antiseptic prisons). The functions of lymphocytes are so diverse and complex that they are beyond the scope of this text (and the scope of the author, it must be admitted). What follows are a few general remarks concerning examination of lymphocytes in peripheral blood.

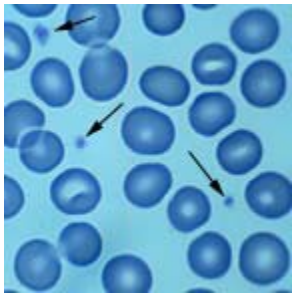
After neutrophils, lymphocytes are the most numerous of the circulating leukocytes. The normal range of the lymphocyte count is 1000 - 4800/μL. Their life span may vary from several days to a lifetime (as for memory lymphocytes). Unlike neutrophils, monocytes, and eosinophils, the lymphocytes 1) can move back and forth between the vessels and the extravascular tissues, 2) are capable of reverting to blast-like cells, and 3) when so transformed, can multiply as the immunologic need arises.

In normal people, most of lymphocytes are small, innocent-looking round cells with heavily "painted-on" nuclear chromatin, scant watery cytoplasm, and no granules. A small proportion of normal lymphs are larger and have more opaque, "busy-looking" cytoplasm and slightly irregular nuclei. Some of these have a few large, dark blue granules, the so called "azurophilic granules." It has been maintained that these

granulated cells are T-gamma cells (i.e., T-cells that have a surface receptor for the IgG Fc region) or natural killer (NK) null-cells. Other phenotypes of lymphocytes are not recognizable as such on the routine, Wright-stained smear and require special techniques for identification.

When activated by whatever means, lymphocytes can become very large (approaching or exceeding the diameter of monocytes) and basophilic (reflecting the increased amount of synthesized cytoplasmic RNA and protein). The cytoplasm becomes finely granular (reflecting increased numbers of organelles), and the nuclear chromatin becomes less clumped (the better to transcribe you with, my dear!). Such cells are called "transformed lymphocytes," "atypical lymphocytes," or "viral lymphocytes" by various votaries of blood smears. Although such cells are classically associated with viral infection (particularly infectious mononucleosis), they may also be seen in bacterial and other infections and in allergic conditions. A morphologic pitfall is mistaking them for monocytes (a harmless mistake) or leukemic blasts (not so harmless).

Platelets



The main thing to remember about platelets is to look for them first! A typical tyro maneuver is to study a blood smear for an hour looking for some profound hematological abnormality, never to realize there is nary a platelet in sight. It is therefore necessary to discipline yourself to first check for a normal number of platelets when sitting down with a slide, before being seduced by the midnight beauty of the basophil's alluring granules or the monocyte's monolithic sovereignty. The normal platelet count is 133 - 333 x 10^3/μL.

Platelets are counted by machine in most hospital labs and by direct phase microscopy in smaller facilities. Since platelets are easily mistaken for garbage (and vice versa) by both techniques, the platelet count is probably the most inaccurate of all the routinely measured hematologic parameters. Actually, you can estimate the platelet count fairly accurately (up to an absolute value of about 500 x 10^3/μL) by multiplying the average number of platelets per oil immersion field by a factor of 20,000. For instance, an average of ten platelets per oil immersion field (derived from the counting of ten fields) would translate to 200,000/μL (10 x 20,000). Abnormal bleeding generally does not occur unless the platelet count is less than 30,000/μL, if the platelets are functioning properly. Screening for proper platelet function is accomplished by use of the bleeding time test.

Other cells in peripheral blood

Plasma cells sometimes appear in the peripheral blood in states characterized by reactivity of lymphocytes. Old time hematologists often maintain that the cells that look exactly like plasma cells on the smear are really "plasmacytoid lymphs," and it is usually nonproductive to argue this point with them. **Endothelial cells** occasionally get scooped up into the phlebotomy needle during blood collection and show up on the slide. They are huge and tend to be present in groups. **Histiocytes**, complete with pseudopodia and phagocytic vacuoles, may appear in states of extreme reactivity, especially in septic neonates. **Nucleated red cells** may also be seen in small numbers in the peripheral blood of newborns; however, in adults, even a single nucleated rbc on the slide is abnormal, indicating some sort of serious marrow stress, from hemolytic anemia to metastatic cancer. **Myeloblasts** are always abnormal and usually indicate leukemia or an allied neoplastic disease. Rarely they may be seen in non-neoplastic conditions, such as recovery from marrow shutdown (aplasia). Later stages of myeloid development (promyelocyte, myelocyte, metamyelocyte) may be represented in the peripheral blood in both reactive states and leukemias.

Bone marrow examination

This is one of the most common biopsy procedures performed on both outpatients and the hospitalized. Two types of specimens are generally obtained, the aspirate and the core biopsy. The site of biopsy is usually the posterior iliac crest (via the posterior superior iliac spine) in adults and the anterior tibia in children, although other sites are available. After local anesthesia is applied to the periosteum and overlying skin, a small needle (usually the "University of Illinois needle") is introduced (or crunched actually) into the medullary space through a small skin incision. About 0.5 mL of marrow material is aspirated and smeared onto several glass slides and stained with a stain identical or similar to the Wright stain used on peripheral blood. Some material usually remains in the syringe where it is allowed to clot. It is then fished out of the syringe, processed like all other biopsy tissue, embedded in paraffin, sectioned, and stained with hematoxylin/eosin and other selected stains. The core biopsy, generally performed after the aspirate is done, is taken with a larger, tapered needle, typically the "Jamshidi needle." This yields a core of bone (similar to a geologic core sample) which is fixed, decalcified, processed, and sectioned. The H&E-stained core biopsy and aspirate clot sections are best for assessment of marrow cellularity and the presence of metastatic neoplasms or granulomas. The Wright-stained aspirate smears are best for studying the detailed cytology of hematopoietic cells.

The bone marrow biopsy procedure produces some pain for the patient, since it is impossible to anesthetize the inside of bone. The level of pain ranges from mild discomfort to agony, depending on the individual's pain threshold and level of apprehension. Some physicians elect to precede the biopsy with a benzodiazepine or other minor tranquilizer. Generally the aspiration action produces much more pain than the core biopsy.

For a procedure that involves invasion of bone, the marrow biopsy is remarkably free of complications. Bleeding and infection may occur but are rare, even in severely thrombocytopenic and immunosuppressed patients. It is highly recommended that med students learn how to perform this useful procedure during the clinical years of their training.