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Blood and Bone Marrow

M1 – Immunology Sequence
J. Matthew Velkey, Ph.D.
Learning Objectives

Reading assignment: Ross and Pawlina, Ch. 10 (Blood and Hemopoiesis)

1. Be able to recognize all of the formed elements found in peripheral blood by light and electron microscopy.
2. Know the approximate abundance and life span of the formed elements.
3. Understand the functions of major plasma proteins and all of the formed elements.
4. Be familiar with the general process of hematopoiesis.
5. Describe the organization of the bone marrow.
6. Be able to recognize megakaryocytes in the bone marrow and understand their function in platelet production.
Functions of the Blood

1. To transport nutrients, oxygen, wastes, and carbon dioxide to and from the tissues.

2. To convey hormones, cytokines, chemokines, and other soluble regulatory molecules.

3. To transport leukocytes and antibodies through the tissues.

4. To maintain homeostasis.
Contents of 1 μl of Peripheral Blood

Plasma (54%)

Formed Elements (46%)
- 200,000-400,000 platelets (1%)
- 5,000-9,000 leukocytes
- 5,000,000-10,000,000 erythrocytes (45%)
## Major Plasma Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Maintain colloid osmotic pressure;</td>
</tr>
<tr>
<td>Globulins</td>
<td>Transport metal ions, protein-bound</td>
</tr>
<tr>
<td>$\alpha$ and $\beta$</td>
<td></td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Antibodies for host defense</td>
</tr>
<tr>
<td>Complement proteins</td>
<td>Destruction of microorganisms</td>
</tr>
<tr>
<td>Clotting factors</td>
<td>Formation of blood clots</td>
</tr>
<tr>
<td>Plasma lipoproteins</td>
<td>Transport of triglycerides and</td>
</tr>
</tbody>
</table>
Cells of the blood

- **Erythrocytes** (red blood cells, RBC)
- **Platelets** (thrombocytes)
- **Leukocytes** (white blood cells, WBC)
  - **Granulocytes** (with specific granules)
    - Neutrophil (~60% of WBC)
    - Eosinophil (~4% of WBC)
    - Basophil (<1% of WBC)
  - **Agranulocytes** (without specific granules)
    - Lymphocyte (B-cell, T-cell) (~27% of WBC)
    - Monocyte (~8% of WBC)
FYI, blood smear procedure

- The procedure for making a blood smear is shown at left.

- After the smear is made, it is air-dried and then stained. Common stains are Wright's stain and Giemsa stain. The stains generally include two or more dyes, one of them a basic dye (often methylene blue) and another an acidic dye (usually eosin). Reddish-blue azures are formed when methylene blue is oxidized (metachromasia). Cells usually stain pink/red with acidic dye and nuclei stain purple/black with basic dye, while specific granules stain characteristically.

- Remember that the cells you see in a blood smear have not been sectioned. Instead you are seeing whole cells dried down on the glass.

Human blood smear, with RBCs, WBCs and platelets

- RBC
- Platelets
- Lymphocyte
- Neutrophil
Erythrocyte (red blood cell, RBC)

1. **Life span in blood:** About 120 days.

2. **Size and shape:**
   - biconcave disk, 8 µm diameter, 2µm at thickest point, 1 µm at thinnest
   - shape maintained by a cytoskeletal complex inside the plasma membrane (involving spectrin, actin and other components)
   - flexible: RBC’s normally bend to pass through small capillaries

3. **LM appearance in smear:** Pink circle with light center (center is thinner because of the biconcave shape). No nucleus.

4. **TEM appearance:** Solid dark gray cytoplasm, because of highly concentrated hemoglobin.

5. **Function:**
   - Transport of oxygen and carbon dioxide
     - bound to hemoglobin (oxyhemoglobin and carboxyhemoglobin)
     - majority of CO₂ transported as HCO₃⁻
   - pH homeostasis
     - carbonic anhydrase: CO₂ + H₂O → HCO₃⁻ + H⁺
     - band 3 membrane protein: exchanges HCO₃⁻ for extracellular Cl⁻
RBCs, scanning electron microscopy
Red blood cells in a blood smear

Mizoguti slide collection (J). J-199.
RBC, transmission electron microscopy

RBC

Platelet
RBC Cytoskeleton and Membrane-Associated Proteins

- Hereditary spherocytosis: defective spectrin; RBCs are fragile and destroyed in spleen leading to anemia.
- A,B,O blood antigens: antigenic carbohydrate chains on extracellular domain of glycophorins
- Rh antigen: multipass integral membrane protein (similar to band 3), also comprises a blood group

Platelets (thrombocytes)

1. **Life Span:** about 10 days

2. **Shape, size, and origin:** Small, biconvex disks, 2-3 μm in diameter. Non-nucleated cell fragments derived from cytoplasm of a very large cell, the megakaryocyte, in bone marrow. Platelets have a life span of about 10 days.

3. **LM appearance in smears:** Small basophilic fragments, often appearing in clusters.

4. **TEM appearance:** The platelet is bounded by a plasma membrane, and has a bundle of microtubules around the margin of the disk (which maintains the disk shape). There are three types of granules, containing fibrinogen, plasminogen, thromboplastin and other factors for clotting. There are also membrane tubules and glycogen.

5. **Function:** Platelets initiate blood clots.
Transmission electron micrographs of a platelet seen in cross section (above) and in a section in the plane of the disk (below).
Cutaway diagram of a platelet

1. Peripheral microtubule bundle (maintains shape)
2. Actin and myosin (clot contraction)
3. Organelles facilitate clotting:
   - Mitochondria for ATP production
   - Granules contain clotting factors
   - Dense tubular system sequesters Ca^{++} for signaling (similar to SR in skeletal muscle)
   - Open canalicular system facilitates signaling and secretion

Platelets and blood clot formation

When a blood vessel wall is damaged, factors from the damaged endothelial cells and the ECM induce the clotting cascade. Platelets aggregate and release proteins for clot formation and resolution:

1. Vasoconstriction – via release of serotonin
2. Further platelet aggregation – mediated via thromboxane A$_2$ and ADP
3. Fibrin polymerization – initiated by thromboplastin and free Ca$^{++}$
4. Clot contraction – via actin, myosin, and ATP released into the matrix of the clot
5. Clot resolution – platelet plasminogen activator (pPA, converts plasminogen into active fibrinolytic plasmin)
6. Tissue repair – platelet derived growth factor (PDGF, stimulates smooth muscle and fibroblast proliferation)
Neutrophil (polymorphonuclear leukocyte)

1. Life Span: < 1 week

2. Granulocyte with specific and non-specific granules

   **Specific granules**
   - Type IV collagenase (aids migration)
   - Lactoferrin (sequesters iron)
   - Phospholipase A\textsubscript{2} (leukotriene synthesis)
   - Lysozyme (digests bacterial cell wall)

   **Non-specific granules (lysosomes)**
   - Lysozyme
   - Acid hydrolase
   - Myeloperoxidase
   - Elastase

3. LM appearance in smear: About 9-12 µm in diameter (thus larger than RBC). Nucleus long and multi-lobed (usually 2-4 lobes).

4. Cytoplasm has small, neutrally stained specific granules. Non-specific granules are azurophilic.

5. TEM appearance: Multi-lobed nucleus and numerous specific granules and lysosomes (=azurophilic granules in LM).

6. Function: Primarily antibacterial
   - Neutrophils leave the blood and follow chemotaxic signals to sites of wounding or other inflammation, and phagocytose foreign agents such as bacteria. Pus is composed largely of dead neutrophils.
Two neutrophils in a blood smear

**LM appearance in smear:** About 9-12 µm in diameter (thus larger than RBC). Nucleus long and multi-lobed (usually 2-4 lobes). Cytoplasm has small, neutrally stained specific granules. Non-specific granules are azurophilic.
Neutrophil, transmission electron micrograph

**TEM appearance:**
Multi-lobed nucleus and numerous specific granules and lysosomes (=azurophilic granules in LM).
Extravasation via diapedesis

- Selectin-selectin receptor interaction causes neutrophil to slow & roll along surface.
- Chemokines from endothelium leads to expression of integrins & immunoglobulin family adhesion molecules on neutrophil cell membrane.
- Neutrophil firmly attached to vessel wall & extends pseudopod into vessel wall.
- Vascular permeability mediated by heparin & histamines released by mast cells/basophils.
- Once in connective tissue, neutrophils respond to chemoattractants & migrate to injury site.
Neutrophil antibacterial activity

1. Chemotaxis and migration (chemokine synthesis and matrix proteolysis)
2. Phagocytosis and bacterial destruction
   - Digestion via lysozymes
   - Production of reactive oxygen compounds (respiratory burst)
     \[ \text{O}_2 \xrightarrow{\text{NADPH oxidase}^*} \text{O}_2^- \xrightarrow{\text{superoxide dismutase}} \text{H}_2\text{O}_2 \xrightarrow{\text{myeloperoxidase}} \text{HOCl} \]
   - Iron sequestration via lactoferrin
3. Release factors to increase inflammatory response (and increase neutrophil production)

*deficiency increases risk of persistent bacterial infections
Eosinophil

1. **Life Span:** < 2 weeks
2. **Granulocyte with specific and non-specific granules**
   - **Specific granules**
     - Major basic protein
     - Eosinophilic cationic protein
     - Neurotoxin
     - Histaminase
   - **Non-specific granules (lysosomes)**
     - Lysozyme
     - Acid hydrolase
     - Myeloperoxidase
     - Elastase
3. **LM appearance in smear:** About 10-14 µm in diameter. Bilobed nucleus. The cytoplasm has prominent pink/red specific granules (stained with eosin dye). If the smear is not stained properly, the granules may be brownish.
4. **TEM appearance:** The specific granules are ovoid in shape, and contain a dark crystalloid body composed of major basic protein (MBP), effective against parasites. The rest of the granule contains other anti-parasitic substances. The cytoplasm also contains lysosomes (=azurophilic granules).
5. **Function:**
   - Anti-parasitic activity
   - Mediators of inflammatory/allergic responses in tissues
     - Inactivate leukotrienes and histamine secreted by basophils
     - Engulf and sequester antigen-antibody complexes
     - Inflammatory stimulus increases production/release of eosinophils from bone marrow, whereas inflammatory suppression decreases eosinophil numbers in peripheral blood.
   - But, they also secrete PRO-inflammatory chemokines AND they can degranulate inappropriately to cause tissue damage (as in reactive airway disease)
**Eosinophil in a human blood smear**

LM appearance in smear: About 10-14 µm in diameter. Bilobed nucleus. The cytoplasm has prominent pink/red specific granules (stained with eosin dye). If the smear is not stained properly, the granules may be brownish.
Eosinophil, transmission electron microscopy

TEM appearance: The specific granules are ovoid in shape, and contain a dark crystalloid body composed of major basic protein (MBP), effective against parasites. The rest of the granule contains other anti-parasitic substances and histaminase. The cytoplasm also contains lysosomes (=azurophilic granules).
Basophil

1. **Life Span:** 1-2 years (?)

2. **Granulocyte with specific and non-specific granules**
   - **Specific granules**
     - Histamine
     - Heparin
     - Eosinophil chemotactic factor
     - Phospholipids for synthesis of leukotrienes, e.g. slow-reacting substance of anaphylaxis (SRS-A)
   - **Non-specific granules (lysosomes)**
     - Lysozyme
     - Acid hydrolase
     - Myeloperoxidase
     - Elastase

3. **LM appearance in smear:** About 8-10 µm in diameter. The cytoplasm contains large, purple/black specific granules (stained with the basic dye) that are larger but not as numerous as those of eosinophils. The nucleus is usually bilobed, but usually is partially obscured by granules, which can lie over it.

4. **TEM appearance:** The specific granules vary in size and shape, and have occasional myelin figures (usually formed from phospholipids). The cytoplasm also has some lysosomes (=azurophilic granules).

5. **Function:** Allergies and anaphylaxis (hypersensitivity reaction)
   - Binding of antigens to membrane-bound IgE antibodies induces degranulation of specific granules, which leads to allergic reaction.
   - In hypersensitivity reaction, widespread vasodilation (arteriolar) and vessel leakiness induce circulatory shock. Bronchial spasms cause respiratory insufficiency; combined effect is anaphylactic shock.

6. **Similarity to tissue mast cells:** Tissue mast cells also have IgE receptors and similar (though not identical) granule content. Mast cells and basophils have a common precursor in bone marrow.
Comparison of basophil and eosinophil in a blood smear

Basophil

Eosinophil
TEM appearance: The specific granules vary in size and shape, and have occasional myelin figures (usually formed from phospholipids). The cytoplasm also has some lysosomes (=azurophilic granules).
Lymphocyte

1. Life Span: variable (few days to several years)
2. LM appearance in smear: Small lymphocyte (about 90% of lymphocytes you will see) are ~8 µm in diameter, while large lymphocytes may be up to about 15 µm. Round, dense nucleus (abundant heterochromatin). The cytoplasm of a small lymphocyte is a narrow rim around the nucleus, and when well stained is pale blue. T-lymphocytes and B-lymphocytes cannot be distinguished in a smear.

3. TEM appearance: The cytoplasm doesn't appear to be very active, containing mainly mitochondria and free ribosomes.

4. Function: Cellular and humoral immunity (more detail in the lecture and lab on lymphatic system histology). In general:
   - B-lymphocytes (B-cells): may differentiate into tissue plasma cells which make antibodies. Some B-cells become memory cells.
   - T-lymphocytes (T-cells): cytotoxic T cells and helper T cells.
Small lymphocyte in a blood smear

LM appearance in smear: Small lymphocyte (about 90% of lymphocytes you will see) are ~8 µm in diameter, while large lymphocytes may be up to about 15 µm. Round, dense nucleus (abundant heterochromatin). The cytoplasm of a small lymphocyte is a narrow rim around the nucleus, and when well-stained is pale blue.
**LM appearance in smear:** Small lymphocytes (about 90% of lymphocytes you will see) are ~8 µm in diameter, while large lymphocytes may be up to about 15 µm with ovoid, dense nuclei (abundant heterochromatin).
TEM appearance: The cytoplasm doesn't appear to be very active, containing mainly mitochondria and free ribosomes.
Tissue plasma cells (derived from B-lymphocytes)
Monocyte

1. **Life Span:** few days in blood, several months in connective tissue
2. **LM appearance in smears:** About 16 µm in smears, thus the largest leukocyte. Large, eccentric nucleus either oval, kidney-shaped or horseshoe-shaped, with delicate chromatin that is less dense than that of lymphocytes. Pale cytoplasm, often grayish, may contain occasional stained granules (lysosomes = azurophilic granules). Large lymphocytes may resemble monocytes, but the lymphocyte nucleus is usually more dense.
3. **TEM appearance:** Cytoplasm contains mitochondria and some small lysosomes.
4. **Function**
   - Migrate into tissues and constitute mononuclear phagocyte system that help destroy foreign bodies and maintain or remodel tissues
     - Tissue macrophages
     - Kupfer cells (liver)
     - Osteoclasts (bone)
     - Dust cells (lungs)
     - Microglia (brain)
   - Mediate inflammatory response
   - Antigen presenting cells: Dendritic Cells, Langerhans cells
Monocyte in a blood smear

**LM appearance in smears:** About 16 µm in smears, thus the largest leukocyte. Large, eccentric nucleus either oval, kidney-shaped or horseshoe-shaped, with delicate chromatin that is less dense than that of lymphocytes. Pale cytoplasm, often grayish, may contain occasional stained granules (lysosomes = azurophilic granules). Large lymphocytes may resemble monocytes, but the lymphocyte nucleus is usually more dense.
Monocyte, transmission electron microscopy

TEM appearance: Cytoplasm contains mitochondria and some small lysosomes.
Blood cell development (hematopoiesis = hemopoiesis)

1. Normally occurs in red bone marrow in adult (also spleen & liver, if necessary)
   Phases: mesoblastic (yolk sac, 2 wks) → hepatic (6 wks) → splenic (12 wks) → myeloid (marrow, 24 wks)
   * Erythrocytes still have nuclei; leukocytes do not appear until 8 wks

2. Mitotic stem and progenitor cells undergo increasing lineage restriction to produce committed precursors.

3. Precursors undergo cell division and differentiation into mature cells.

4. Maturation involves (note exceptions for megakaryocytes below):
   - decrease in cell size*
   - shutting down transcription (nucleoli disappear and chromatin condenses)*
   - adoption of morphological characteristics specific to that lineage.
     - Future granulocytes produce specific and non-specific granules, and then shape their nucleus.
     - Future monocytes produce non-specific granules and shape their nucleus.
     - Future small lymphocytes decrease their size and enter the blood, but then undergo extensive further maturation at another site (T-cells in the thymus, and B-cells in the "bursa equivalent" –to be discussed in immune system lecture).
     - Future erythrocytes fill cytoplasm with hemoglobin, synthesized on free polysomes (ribosomes on mRNA), and eventually extrude their nucleus.

5. Mature cells enter marrow sinus; immature cells in peripheral blood typically indicates disease.

* Megakaryocytes develop into large polyploid cells that remain transcriptionally active and extrude platelets as cytoplasmic fragments directly into marrow sinus.
Lineage Restriction

Junqueira's Basic Histology, 10th edition, page 250.
Cellular Changes during Myeloid Differentiation

**Mitotic Cell**
- Myelocyte

**Postmitotic Cells**
- Metamyelocyte
- Band Cell
- Mature Neutrophil

**Nuclear Changes**
- Large, euchromatic, transcriptionally active nucleus → Smaller, euchromatic, less transcriptionally active nucleus → Condensed, heterochromatic, transcriptionally inactive nucleus

**Cytoplasmic Changes**
- Basophilic cytoplasm, active synthesis of specific and nonspecific granules → Reduced basophilia, granule maturation → Pale bluish-pink cytoplasm, mature granules
Changes during Erythroblast Differentiation

Mitotic Cells

Proerythroblast

Early Erythroblast

Middle Erythroblast

Late Erythroblast

Erythrocyte (Reticulocyte)

Postmitotic Cells

Nuclear Changes-
Large, euchromatic, and transcriptionally active → Smaller, heterochromatic, and transcriptionally inactive → Absent

Cytoplasmic Changes-
Basophilic with abundant RER → Polychromatic (basophilic and acidophilic) with abundant RER and hemoglobin → Acidophilic, with abundant hemoglobin
EM of developing erythrocyte, showing polyribosomes and hemoglobin in cytoplasm
Extrusion of nucleus from a developing erythrocyte in bone marrow, EM
Reticulocytes (somewhat immature RBCs) in blood smear, cresyl blue stain

Residual ribosomes in cytoplasm are basophilic. Number of reticulocytes in peripheral blood reflects status of erythropoiesis – generally increased by anemia and hypoxia.
Finger, bone marrow in phalanges
Section of bone marrow, LM

Megakaryocytes

Sinus

RBCs

Bone

Marrow

Dr. A. Kent Christensen, histological slide from Carolina Biological Supply Co.
Marrow sinuses are sinusoidal, discontinuous capillaries. Mature cells enter the sinuses and are conveyed to the systemic circulation via nutrient veins.
Diagram of bone marrow sinus showing intravasation of blood cells and megakaryocyte releasing platelets into bloodstream

Image of megakaryocyte intravasation removed

Megakaryocytes in bone marrow produce blood platelets

- **LM appearance:** A huge cell, up to 50 µm in diameter. Its long nucleus has several lobes (the nucleus is polyploid and can be up to 64N). The cytoplasm is pale pink/red, without visible granules. In bone marrow, megakaryocytes are situated adjacent to a marrow sinus (large capillary), although this may not be obvious in tissue sections.

- **TEM appearance:** Particularly striking in the cytoplasm are many curved white lines that are the platelet demarcation channels, membrane-bound spaces forming the boundaries between future platelets. The cytoplasm also contains granules of various sizes, that will be in the platelets.

- **Function:** Megakaryocytes produce blood platelets by fragmentation of their cytoplasm, extending cell processes through the endothelium of a marrow sinus, and releasing clusters of immature platelets into the blood, to become mature platelets.
Megakaryocyte, LM section

**LM appearance:** A huge cell, up to 50 µm in diameter. Its long nucleus has several lobes (the nucleus is polyploid and can be up to 64N). The cytoplasm is pale pink/red, without visible granules. In bone marrow, megakaryocytes are situated adjacent to a marrow sinus (large capillary), although this may not be obvious in tissue sections.
Electron micrograph of megakaryocyte, source of platelets

Particularly striking in the cytoplasm are many curved white lines that are the platelet demarcation channels, membrane-bound spaces forming the boundaries between future platelets. The cytoplasm also contains granules of various sizes that will be incorporated into the platelets.
EM detail of megakaryocyte cytoplasm

Will be extruded as a platelet
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Slide 43: Source Undetermined
Slide 48: Dr. A. Kent Christensen, histological slide from Carolina Biological Supply Co.
Slide 49: Source Undetermined; Gray's Anatomy
Slide 53: Fawcett's Concise Histology, 2nd ed., page 59
Slide 54: Fawcett's Concise Histology, 2nd ed., page 60.