Bone Marrow, Blood, and Lymph Node Histology

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Objectives

• Become familiar with the histology of:
  • Blood
  • Bone marrow
  • Lymph node
  • Spleen

• Understand
  • Development and regulation of hematopoiesis
  • Role of lymph nodes in immune regulation

Blood

• Fluid connective tissue
• Cells (formed elements)
  • Red blood cells
  • White blood cells
  • Platelets
• Extracellular matrix
  • Blood plasma serves as a solvent for a variety of solutes
  • Protein-rich matrix

Erythrocytes

• Greatest cellular constituent of the blood
• Biconcave discs
• Lack nucleus
• Contain Hemoglobin
  • Transport O₂, CO₂
  • Acid-base balance

Erythropoiesis

Marrow Maturation

<table>
<thead>
<tr>
<th>Marrow Maturation</th>
<th>Early</th>
<th>Intermediate</th>
<th>Late</th>
<th>Blood</th>
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</thead>
<tbody>
<tr>
<td>CTU/G, normoblast</td>
<td>BL, proerythroblast</td>
<td>Polychromat. Normoblast</td>
<td>Reticulocyte</td>
<td>Erythroblast</td>
</tr>
<tr>
<td>Cell size (μm³)</td>
<td>100</td>
<td>70</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>Mitochondrion:RNA ratio</td>
<td>2.5</td>
<td>2.1</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

Erythropoiesis

Proerythroblast

Basophilic erythroblast

Polychromat. erythroblast

Reticulocyte

Normoblast
Red Blood Cell - Case

- 6 y.o. African American male
- 1 day severe leg and foot pain. Viral upper respiratory infection for 3 days.
- No current or past medical problems
- Family history of anemia
- What’s next?

Red Blood Cell - Case

- Complete blood count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>12,000 (nl 5,000-10,000)</td>
</tr>
<tr>
<td>% neutrophils</td>
<td>38%</td>
</tr>
<tr>
<td>% lymphocytes</td>
<td>60%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>20% (nl 37-42%)</td>
</tr>
<tr>
<td>Platelets</td>
<td>460 (nl 350-500)</td>
</tr>
</tbody>
</table>

Red Blood Cell - Case

- Normal
- Patient

Anemia

- Blood loss
- Decreased RBC production - Anemia will ultimately result if the rate of RBC production is less than that of RBC destruction.
  - Lack of nutrients, such as iron, B12, or folate.
  - Bone marrow disorders or bone marrow suppression
  - Low levels of trophic hormones which stimulate RBC production (EPO-renal failure, thyroid hormone, androgens.
  - Other diseases (anemia of chronic disease).
- Increased RBC destruction
  - Inherited hemolytic anemias (eg, hereditary spherocytosis, sickle cell disease, thalassemia major)
  - Acquired hemolytic anemias (eg, Coombs’-positive autoimmune hemolytic anemia, thrombotic thrombocytopenic purpura-hemolytic uremic syndrome, malaria)

Sickle Cell Anemia (1)

- Valine for glutamic acid substitution in β-globin chain
- Leads to αβ(s)2 hemoglobin tetramers
  - Poorly soluble compared to normal αβ2 Hb
  - Polymerize at low pO2 and pH leading to sickle red blood cells
  - Results in occlusion of venous blood vessels
- Anemia from rapid degradation of sickle red blood cells

Sickle Cell Anemia (2)
**White Blood Cells**

- 5-10 x 10^6 WBC/ml in adults
- Defense against foreign substances
- Two pools of WBC in blood
  - Circulating pool (included in WBC count)
  - Non-circulating pool – marginating pool or group of cells that rest against blood vessel walls
- Five types, classified by:
  - Nuclear morphology
    - Polymorphonuclear
  - Presence or absence of cytoplasmic granules and their staining properties (if present)

**Neutrophils (PMNs)**

- 40-75% of circulating WBC
- Life span is 2-3 days
- Diameter is 10-15um
- Single multi-lobed heterochromatic nucleus

**Neutrophils (PMNs)**

- Primary granules (lysosomal granules)
  - Stain azurophilic
  - Contain lysozymes, defensins, elastase, acid phosphatase and myeloperoxidase
- Secondary granules (specific granules)
  - Specific to neutrophils
  - Stain pink or neutral
  - Contain lactoferrin, lysozyme, phagocytins, collagenases and cytochrome b
- Play a major role in protection against bacterial and fungal infections

**Basophils**

- Least numerous mature WBC
- Bi-lobed nucleus
- Granules
  - Large and abundant
  - Stain with basic dyes
- Contents
  - Heparin sulfate
  - Histamine
  - Slow reacting substance of anaphylaxis
- Mediate allergic responses
- Express a receptor for IgE
- Antigen binds to and crosslinks the IgE molecules
- Substances released from granules play a major role in the hypersensitivity reactions
- May lead to cardiovascular and respiratory collapse (anaphylaxis)

**Eosinophils**

- Bi-lobed nucleus
- Granules
  - Large refractile red or orange
  - Major basic protein
  - Toxic to parasites
  - Vasoactive agents
  - Agents that limit inflammatory response
**Eosinophils**
- **Immunity**
  - Undergo chemotaxis in response to bacterial products and complement components
  - Ingest and destroy antigen-antibody complexes
  - Important in defense against parasites
- **Mediate allergic response**
  - Express receptors for IgE
- **Attenuate inflammatory responses**
  - Preferentially attracted by substances released from basophils and mast cells
  - Release of histaminase moderates the potentially deleterious effects of the vasoactive agents

**Monocytes**
- **Life span of months to years**
- 3-8/100 WBCs
- **Nucleus**
  - Large, eccentrically placed
  - Stains deep blue to purple
  - Indention becomes more pronounced with maturity
- **Cytoplasm**
  - Ground glass appearance
  - Blue/gray
  - Azurophilic granules

**Monocytes**
- Little function in circulating blood
- Migrate to peripheral tissues where they assume the role of macrophages
- Respond to presence of necrotic material and invading microorganisms
  - Large content of lysosomal enzymes
  - Engulf and destroy tissue debris and foreign material
  - Present antigens to adaptive immune system

**Myeloid development**
- Myeloblast
- Promyelocyte
- Myelocyte
- Metamyelocyte
- Band Neutrophil
- Segmented Neutrophil

**Lymphocytes**
- **Variable size** (6-18μm)
  - Smallest are quiescent
- **Nucleus**
  - Intensely stained
  - Slightly indented
  - Spherical in shape
- **Cytoplasm** appears as a pale blue rim around nucleus

**Lymphocytes**
- Main functional cell of adaptive immune system
- Most found in blood or lymph are “recirculating immunocompetent cells”
  - T Lymphocytes (thymus-dependent)
    - Have a long life span
    - Involved in cell mediated immunity
  - B Lymphocytes (B cells)
    - Variable life span
    - Involved in the production of circulating antibodies
  - Indistinguishable in blood smears or tissue section
Platelets

- Life span of 10 days
- 2-4μm in diameter
- Exhibit an intensely stained core
- α-lysosomal granules: red-violet - azurophilic
- Clotting factors: fibrinogen, von Willebrand factor, thrombospondin, and fibronectin, adenine nucleotides
- Growth factors: platelet derived growth factor = PDGF
- Chemotactic factors
- Vasoactive substances: serotonin

Megakaryocytes

Hematopoiesis

Regulate peripheral blood cell types and numbers

- Maintain adequate numbers of blood cell (homeostasis)
- Proper number of specialized cell types
- Increase cell production with changing needs
  - Granulocytes: bacterial infections
  - Eosinophils: parasitic infections
  - Erythrocytes: high altitude
- Prevent excessive cell production (leukemia)

Bone marrow histology

- Yellow Marrow: Mostly fat cells
- Red Marrow: Formation of blood cells
- Bone marrow cellularity = 100 - age

Bone marrow histology

- Hematopoietic cords
- Hematopoietic cells
- Stroma
- Microenvironment
- Vasculature
  - Sinusoidal capillaries
  - Endothelial cells
  - Reticular (adventitial cells)

Hematopoietic development

Mesoblastic phase (1-2 mos)
- Blood islands of the yolk sac
- Cells produce hemoglobin and become nucleated RBC's

Hepatic phase (2-5 mos)
- Normal RBC's produced in reticuloendothelial system (liver, spleen, thymus, etc.)
- Initial production of myeloid and lymphoid cells

Myeloid phase (5-9 mos)
- Bone marrow is primary site of blood cell production

Kids and young adults (birth - 20 years)
- Hematopoiesis in both long bones and axial skeleton

Older adults (20 years - )
- Most hematopoiesis in axial skeleton
Hematopoiesis

Stem cells

Committed progenitors

Differentiated cells

Regulation of HSC

Cells
- Bone marrow stromal cells
- Osteoblasts
- Endothelial cells

Extracellular matrix
- Fibronectin, collagen, vitronectin, tenascin

Molecules involved
- Cytokines - IL-3, SCF, Tie2
- Chemokines - CXCR4/SDF-1
- Morphogens - Notch, Wnt, BMP
- Adhesion molecules - VLA4, LFA-2, E-selectin, ICAM-1, VCAM-1

Regulation of HSC

Function of stem cell niche
- Cellular homing
- Protection and survival of HSC
- Maintain pluripotency (prevent differentiation)
- Regulate self-renewal (asymetrical vs symetrical cell division)

Progenitors
- Symmetrical Division
  - Cells mature
  - Lose some ability to divide

Stem Cells
- Asymmetrical Division
  - One daughter cell matures as the other remains as an exact copy of parent (self-renewal)
  - Maintains stem cell pool

Bone Marrow Case #1

- 28 y.o. female
- 3 months of heavy menstrual bleeding, 2 weeks of progressive fatigue
- No current or past medical problems
- No family history of bleeding

Bone Marrow Case #1

- Complete blood count
  - White blood cells: 800 (nl 5,000-10,000)
  - % neutrophils: 5%
  - % lymphocytes: 95%
  - Hematocrit: 21% (nl 37-42%)
  - Platelets: 35 (nl 350-500)
Bone Marrow Case #1

Normal Patient

Bone Marrow Failure (1)

• Aplastic anemia
  • Acquired - immune destruction of hematopoiesis
    • Induced by drugs
    • Following infections (hepatitis)
  • Inherited -
    • Fanconi Anemia - DNA repair mechanisms
    • Schwachman-Diamond - RNA processing
    • Dyskeratosis congenita - Telomerase

Bone Marrow Case #2

• 75 y.o. male
• 2 months of malaise, fevers for 3 days, no abnormal bleeding
• History of hypertension, smoking
• No family history of cancer or bleeding

Bone Marrow Failure (2)

• Infiltrative - marrow replacement
  • Leukemias
  • Myelodysplastic syndrome
  • Lymphoma
  • Other cancers
  • Fibrosis
  • Nutritional deficiencies - Vit B12
  • Bad infections (viral)

Bone Marrow Case #2

• Complete blood count
  White blood cells 62,000 (nl 5-10,000)
  % neutrophils 1%
  % lymphocytes 1%
  % other 98%
  Hematocrit 18% (nl 37-42%)
  Platelets 15 (nl 350-500)
### Acute Myeloid Leukemia
- Expansion of myeloid cells in bone marrow and/or blood with blocked differentiation
- Arise from normal hematopoietic stem cells
- Many different genetic lesions
  - Chromosomal translocations, genetic instability
  - Clinical decisions based on chromosomal abnormalities

### Lymphoid Anatomy
- Two major functional regions:
  - **Primary immune organs**
    - Sites of initial maturation and development of immune competent cells
    - B cells - bone marrow
    - T cells - thymus
  - **Secondary immune organs**
    - Sites of interaction between antigens and immune cells
    - Antigen driven replication and differentiation into effector cells

### Peripheral (Secondary) Lymphoid Tissue
- **Lymph nodes**
  - Encapsulated
  - Interposed between lymphatic vessels (only)
- **Spleen lymphatic tissue**
  - Encapsulated
  - Interposed between lymph and blood circulation
- **Diffuse lymphatic tissue**
  - Unencapsulated
  - GI (tonsils, Peyer’s patches, appendix); Respiratory tract; Genitourinary
  - Found in the lamina propria where pathogens are likely to invade

### Lymph Node Anatomy
- **Capsule**: Dense, Irregular CT (collagen type I stains red brown)
- **Trabeculae**: Extensions of the capsule
- **Reticular tissue**: Framework (collagen III stains black with the Silver stain)
- **Hilum**: Efferent lymph; entry and exit of blood vessels

### Lymph Node Histology
- **Capsule**: Dense, Irregular CT (collagen type I stains red brown)
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Lymph Node Parenchyma

- **Cortex**
  - Primary, secondary nodules
  - B cells
- **Deep cortex**
  - Paracortex, juxtamedullary cortex, thymus dependent cortex
  - No nodules
  - T cells
- **Medullary cords**
  - Plasma cells synthesize and release antibodies into lymph flowing through the sinuses

Lymph Node Cortex

- **Primary follicle**
  - Naive B cells
  - No germinal center
- **Secondary follicle**
  - Germinal Center
  - Antigen has been encountered
  - Looks pale because of plasmablasts: large euchromatic nuclei
  - Surrounded by T cells

Reactive Germinal Center

- Dark zone
  - Densely packed B lymphocytes separated by reticular cells
- Light zone
  - Immunoblasts/plasmablasts
  - Large cells, central nucleus, prominent nucleolus
  - T cells at periphery
- Mantle zone
  - Naive B and T cells

Medullary Cord and Sinus

- Medullary cord
  - Lymphocytes
  - Plasma cells
  - Blood vessels
  - Macrophages
- Medullary sinus
  - Macrophages

Lymph Flow

- Afferent Lymphatics → subcapsular sinus → trabecular sinuses → medullary sinus → efferent lymphatics

Lymphocyte Trafficking

- Naive B and T lymphocytes home to specific sites within the lymph node via peripheral blood
- B cell homing include:
  - The primary and secondary cortical follicles
  - Medullary cords: plasma cells release immunoglobulins into the efferent lymph
- T cell homing
  - primarily paracortex
**Lymph Node Case #1**

- 8 y.o. female
- "Lump" in neck growing over 3 weeks
- No past medical problems
- No family history of cancer
- Physical exam - enlarged, rubbery, movable 6 cm lymph node in lateral neck

- What next?

**Lymph Node Case #1**

- Lymph node biopsy

**Lymph Node Case #2**

- 58 y.o. male
- "Lump" in neck growing over 2 weeks. Fever for 1 month, night sweats for 2 weeks, 20 lbs weight loss
- No past medical problems
- No family history of cancer
- Physical exam - enlarged, rubbery, movable 3 cm lymph node in lateral neck, 2 cm supraclavicular lymph node on right

- What next?

**Lymphadenopathy (1)**

- **Bacterial** - Localized Streptococcal pharyngitis; skin infections; *Streptococcus*; plague; cat scratch disease; diphtheria; chancroid; rat bite fever; brucellosis; leptospirosis; lymphogranuloma venereum; typhoid fever
- **Viral** - Human immunodeficiency virus; Epstein-Barr virus; herpes simplex virus; cytomegalovirus; mumps; measles; rubella; hepatitis B; dengue fever
- **Mycobacterial** - Mycobacterium tuberculosis; atypical mycobacteria
- **Fungal** - Histoplasmosis; coccidioidomycosis; cryptococcosis
- **Protozoal** - Toxoplasmosis; Leishmaniasis
- **Spirochetal** - Secondary syphilis; Lyme disease

**Cat Scratch Disease**

- Caused by *Bartonella henselae*
- Most cases resolve spontaneously
- May disseminate to organs (liver, spleen)
- Unclear role for antibiotics in localized disease
Lymph Node Case #2

- Lymph node biopsy

Lymphadenopathy (2)

- Cancer - Squamous cell cancer head and neck; metastatic; lymphoma; leukemia
- Lymphoproliferative angioimmunoblastic lymphadenopathy with dysproteinemia
- Autoimmune lymphoproliferative disease - Rosai-Dorfman's disease, hemophagocytic lymphohistiocytosis
- Immunologic - Serum sickness; drug reactions (phenytoin)
- Endocrine - Hypothyroidism; addison's disease

The Spleen

**Functions**

- Site of fetal hematopoiesis
- Reservoir for red blood cells
- Filters blood
- Iron retrieval
- Immune regulation

Spleen

- Capsule
  - Dense connective tissue
- White pulp
  - lymphoid tissue
- Red pulp
  - sinusoids

- White pulp
  - lymphoid tissue
- Red pulp
  - sinusoids
- Red blood cells
- Periarterial lymphoid sheath
  - lymphocytes
Spleen
- Central artery
- Periarterial lymphoid sheath
- Mantle zone
- Trabecular vein

MALT
Mucosal associated lymphoid tissue
- GALT: gut-associated lymphoid tissue
- BALT: bronchial/tracheal-associated lymphoid tissue
- SALT: skin associated lymphoid tissue
- etc...
- Antigen presentation of luminal antigens to MALT by specialized epithelial cells (M cells)
- Primary immunoglobulin isotype produced is IgA (secretory)
- Intraepithelial lymphocytes also play a role in immune surveillance at mucosal surfaces.