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

- Proerythroblast
- Basophilic erythroblast
- Polychromatic erythroblast
- Normoblast
- Reticulocyte

Erythropoiesis

- Marrow Maturation
- Time (h)
- Cell size 100 μm
- Mitochondria RNA content
- Hemoglobin

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

- White blood cells 12,000 (nl 5,000-10,000)
- % neutrophils 38%
- % lymphocytes 60%
- Hematocrit 20% (nl 37-42%)
- Platelets 460 (nl 350-500)

Red Blood Cell - Case

Normal

Patient

Red Blood Cell - Case

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 nl αβ_2 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
    - Mononuclear
  - 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

- 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 (6-18um)**
  - 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
- 2-4um 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**
- [Developmental pathways image]

**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

**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

**Bone marrow histology**
- Hematopoietic cords
- Hematopoietic cells
- Stroma
- Microenvironment
- Vasculature
  - Sinusoidal capillaries
  - Endothelial cells
  - Reticular (adventitial cells)
Hematopoiesis

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 - CXC/R4/SSFR-1
- Morphogens - Notch, Wnt, BMP
- Adhesion molecules - VLA4, LFA-2, E-selectin, ICAM-1, VCAM-1

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

Regulation of Progenitors

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>800 (nl 5,000-10,000)</td>
</tr>
<tr>
<td>% neutrophils</td>
<td>5%</td>
</tr>
<tr>
<td>% lymphocytes</td>
<td>95%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>21% (nl 37-42%)</td>
</tr>
<tr>
<td>Platelets</td>
<td>35 (nl 350-500)</td>
</tr>
</tbody>
</table>
Normal Patient

Bone Marrow Case #1

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

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

Lymph Node Histology

- Capsule: Dense, irregular CT (collagen type I stains red brown)
- Trabeculae are 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 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**
  - Naïve 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

Reacting 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**
  - Naïve B and T cells

Medullary Cord and Sinus

- **Medullary cord**
  - Lymphocytes
  - Plasma cells
- **Medullary sinus**
  - Macrophages

Lymph Flow

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

Lymphocyte Trafficking

- Naïve 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

Lymphadenopathy (1)

- **Bacterial** - Localized Streptococcal pharyngitis; skin infections; *Brucella*, 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; cryptococciosis
- **Protozoal** - Toxoplasmosis; Leishmaniasis
- **Spirochetal** - Secondary syphilis; Lyme disease

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?

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

Hodgkin’s disease

- Characterized by Reed-Sternberg cells
- Derived from B cells
- Affects kids and elderly
- May be caused by EBV
- May be associated with “B” symptoms - fever, night sweats and weight loss
- Highly curable with chemotherapy

Lymphadenopathy (2)

- Cancer - Squamous cell cancer head and neck; metastatic; lymphoma; leukemia
- Lymphoproliferative angioimmunoblastic lymphadenopathy with dysproteinemina
- 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

Splenic Vasculature

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.