Normal hematopoiesis & coagulation

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Objectives

1. To recognize features of normal bone marrow histology and indices of effective hematopoiesis.
2. To understand the fundamentals of how blood clots.
3. To recognize some examples of genetic defects affecting hematopoiesis and coagulation.
4. To contrast loss-of-function and gain-of-function mutations.
Normal hematopoiesis
Multipotential hematopoietic stem cell

- Common myeloid progenitor
  - Megakaryoblast
    - Promegakaryoblast
      - Megakaryocyte
        - Platelets
      - Proerythroblast (Pronormoblast)
        - Basophilic erythroblast
          - Polychromatic erythroblast
            - Normoblast
              - Reticulocyte
                - Erythrocyte
          - Promyelocyte
            - Myelocyte
              - Metamyelocyte
                - Band
                  - Neutrophil
                  - Eosinophil
                  - Basophil
                  - Plasma cell
            - Monoblast
              - Promonocyte
                - Monocyte
                  - Monocyte

- Common lymphoid progenitor
  - Lymphoblast
    - T lymphocyte
    - B lymphocyte
    - Plasma cell
Myeloid Lineage

- Usually accounts for much of the marrow cellularity
- Loose organization where more immature cells tend to be located next to trabecular bone
- Should see complete maturation to bands/neutrophils within the central marrow
Erythroid Lineage

- Usually accounts for 20-20% of cellularity
- Organized as small “islands” of erythroid precursors with a full range of maturation
Megakaryocytes

- Large, polyploid cells with abundant cytoplasm
- Interspersed singly throughout the marrow parenchyma in juxtaposition with marrow sinus spaces, where platelets are shed
Normal Bone Marrow Histology

- % cellularity
describes the ‘non-fat’ space
age dependent (cellularity is roughly equal to 100 – age)

- Cellular composition “trilineage hematopoiesis”
myeloid cells (mostly maturing granulocytes)
erythroid cells (nucleated red cells; M:E ratio of 3-5:1)
megakaryocytes (large, polyploid cells near sinuses)
Clinical Manifestations of Cytopenias

- agranulocytosis/granulocytopenia
  opportunistic infections
- anemia
  fatigue, shortness of breath, pallor
- thrombocytopenia
  bruises, mucosal bleeding, petechiae
Cells in the peripheral circulation

- **Neutrophils**: 50%-70% of leukocytes
- **Lymphocytes**: 25%-35% of leukocytes
- **Eosinophils**: <5% of leukocytes
- **Basophils**: <1% of leukocytes
- **Monocytes**: 3%-9% of leukocytes
- **Erythrocytes and Platelets**
Granulocytes

**Neutrophil**
- tiny, numerous, light staining granules
- multi lobed nucleus, connected by nuclear material
- larger than red blood cells

**Eosinophil**
- large granules, pink/red due to acidity
- two lobes connected by nuclear material

**Basophil**
- purple/blue granules are large and numerous masking the nucleus
- segmented nucleus
NEUTROPHILS (50-70%)  
Main target: **bacteria and fungi**

They are **phagocytes**, capable of ingesting (**phago-** meaning **to eat**) microorganisms or particles.

They also release proteins in **granules** through the process of **degranulation**. The protein contents have antimicrobial properties, e.g., lysozyme, collagenase, NADPH oxidase.

**Histologic Features:**  
4-lobed nucleus, visible cytoplasm, tend to be found in groups (in tissue sections).
EOSINOPHILS (<5%)

Called “eosinophilic” or acid-loving, as they stain bright red with eosin.

Responsible for combating multicellular parasites, and help control processes associated with asthma and allergies.
MONOCYTES (3-9%)

Many monocytes are stored in the spleen (over half), and in response to inflammation signals, move quickly and can divide and differentiate into macrophages and dendritic cells.

Macrophages phagocytose cellular debris and pathogens, and stimulate lymphocytes.

Dendritic cells act primarily as antigen-presenting cells (APC) that activate T lymphocytes.

**Histologic Features:**
Large, horseshoe or irregular nucleus, abundant blue cytoplasm
Monocyte to Macrophage

- Monocytes = large cell with horseshoe shaped nucleus
  - Remain in circulation for ~24 hours before entering peripheral tissue to become a tissue macrophage
- Active macrophages - aggressive, engulf items as large as themselves
  - Histologic Features: 5 to 10 fold larger than monocytes, nucleus is more round, open & pale, and abundant cytoplasm
  - Cytoplasm can contain pigments (i.e. hemosiderin-laden macrophage)
  - Can form sheets in granulomas or become multi-nucleated giant cells
LYMPHOCYTES (25-35%)

Come in three major types:
1) T-cells (Thymus cells)
2) B-cells (Bursa-derived cells)
3) NK Cells (Natural Killer)

T-Cells are responsible for **cell-mediated immunity** (i.e., relating to activation of phagocytes, NK cells, and release of cytokines)

B-Cells are responsible for **humoral immunity** (i.e., relating to creation and secretion of antibodies).
ERYTHROCYTES & PLATELETS (THROMBOCYTES)

Erythrocyte: red blood cells, the principle method of delivery oxygen to body tissues.

The cytoplasm of erythrocytes is full of hemoglobin.

Histologic features:
No nucleus, bright red on H&E, pale pink on Giemsa stain.

Platelets, small irregular shaped cell fragments of megakaryocytes, the primary function of which is to maintain hemostasis, or to stop bleeding.

Histologic features:
No nucleus, not generally appreciated on tissue sections unless clumped, very small, seen on blood smears.
Normal hemostasis
Hemostasis

- Hemostasis is the process that leads to the stopping of bleeding.
- Hemostasis involves blood vessels, platelets, plasma clotting proteins.
- Primary Hemostasis is the response to injury to a blood vessel involving platelets.
- Secondary Hemostasis occurs to fortify primary hemostasis through the activation of clotting proteins to form a insoluble deposition of fibrin.
Primary Hemostasis
Secondary Hemostasis
Hemostasis

- Intricate system maintaining blood in fluid state
  - Reacts to vascular injury to stop blood loss and seal vessel wall
- Involves platelets, clotting factors, endothelium, and inhibitory/control mechanisms
  - Highly developed system of checks and balances
  - Designed to turn on when needed and to stop processes before going too far

**Normal Hemostasis**

*Absence of overt bleeding/thrombosis*

**Bleeding**  **Thrombosis**
Platelets are typically 1-2 micron
The normal PLT count is 150-350,000/ul
One large one above shows how granular they appear.
Platelet- Number, Lifespan and Kinetics

- Normal platelet concentration is 150,000- 350,000/ uL
- Platelet Size 1.5 microns, with a volume of 9 femtoliters
- They circulate in the blood for about 10 days after release from the marrow
Stages of platelet development

Stem cell → commitment → BFU-Mk → CFU-Mk → Immature Mk → Mature Mk → Terminal differentiation

Platelet shedding

All stages are driven by thrombopoietin
Thrombopoietin (TPO)

• Growth factor produced in liver, little from kidney
• Increases production of megakaryocytes
Thrombopoietin Signaling

Thrombopoietin

- Glycoprotein
- 332 amino acids, 95 kDa
- Synthesized mainly in the liver

Thrombopoietin Receptor

- Cytokine receptor
- Homologous to the oncogene in murine myeloproliferative leukemia virus
- Present on megakaryocytes and platelets

- Mpl-binding domain
- COOH terminal domain
Platelets act in Concert with Fibrin Formation to Form a Clot

Fibrinogen

Fibrin – polymerized remains of fibrinogen

Thrombin
Secondary Hemostasis
What is Thrombosis?

- Venous Thromboembolism (VTE) comprises DVT & PE
- Deep vein thrombosis (DVT) is a condition in which a blood clot forms inside a deep vein
- Commonly located in calf or thigh
- Occurs when the blood clot either partially blocks or completely blocks blood flow in the vein
Venous Thromboembolism – Incidence & Significance

• Complications from DVT kill up to 200,000 people per year in the U.S.
• Third most common vascular disease
• Pulmonary Embolism (PE) leading preventable cause of death
  – PE occurs when a blood clot breaks loose from the wall of a vein and travels to the lungs, blocking the pulmonary artery or one of its branches
• PE leading cause of maternal death associated with childbirth
• DVT/PE incidence is unknown, but estimates range from 300,000 to 600,000 (1 to 2 per 1,000, and in those over 80 years of age, as high as 1 in 100) each year in the United States.
VENOUS THROMBOEMBOLISM

Virchow’s triad for venous thromboembolism:

- Reduced Blood Flow
- Vessel Damage
- Change in Blood Components
Venous Thromboembolism

• Complex, multi-causal disease
  – Physiological factors
    • Age, hormonal influence (i.e. pregnancy)
  – Acquired risk factors
    • Cancer, surgery, obesity, trauma, immobility
  – Hereditary (genetic) risk factors
    • Deficiencies in anticoagulation proteins
    • Elevated coagulation proteins
    • Gene mutations preventing function of proteins
Coagulation Cascade

• Vascular damage initiates the coagulation cascade.
• Results in the generation of thrombin at the site of injury.
• Thrombin catalyzes the conversion of fibrinogen to an insoluble fibrin (clot) matrix.
Coagulation Cascade

• Abnormal activation of blood coagulation and/or depressed fibrinolytic activity may lead to the formation of a thrombus (clot).

• In contrast, a defect or deficiency in the coagulation process and/or accelerated fibrinolysis is associated with a bleeding tendency.
Coagulation Cascade

• The cascade scheme is organized into the INTRINSIC and EXTRINSIC pathways, converging into the COMMON pathway.
Extrinsic Pathway

“Tissue Factor Pathway”
Initiated when blood is exposed to TF released from damaged endothelium
  • Tissue Factor (TF), FVII
  • Measured with PT clotting assay
Common Pathway Activation

TF not only activates factor X but also factor IX (nine), also activated in the intrinsic pathway; thrombin also activates factor VIII.

Factor VIIIa and IXa form a complex called tenase, that produces more Xa. Factor V is activated by thrombin into factor Va. Xa and Va bind onto a phospholipid surface and form the prothrombinase complex which very efficiently converts more prothrombin into thrombin.
Abnormalities of hematopoiesis & coagulation
Inherited Defects in Hematopoiesis

A heterogeneous group of genetic disorders that can cause failures in one or several hematopoietic lineages.

- Mutations in thrombopoietin receptor (c-MPL) cause thrombocytopenia (unilineage)
- Mutations in DNA repair proteins cause pancytopenia (multi-lineage effect) **Fanconi anemia**
Clinical presentation: Fanconi Anemia

Usually presents with physical anomalies early in life or with hematologic manifestations within the first decade.

Cytopenias (usually thrombocytopenia followed by progressive aplasia/pancytopenia; affect 90% of patients by age 40).

Incidence: less than 1/100,000
Physical Findings in Fanconi Anemia

Café-au-lait spots & other pigmentation changes (65%)

Short stature (60%)

Upper limb abnormalities (hypoplastic or bifid/supernumerary thumbs most common, 50%)

“Fanconi facies”
Laboratory Findings in Fanconi Anemia
Complementation studies define different types of Fanconi Anemia

Two potential outcomes:

MMC sensitivity phenotype persists (i.e., same “complementation group”), therefore the unknown lesion is presumed to be in FANCA

MMC sensitivity is rescued (i.e., FANCA−/− “is complemented”), therefore the unknown lesion is presumed to be in another gene
Genes Mutated in Fanconi Anemia

- **FANCA**: 16q24.3
- **FANCC**: 9q22.3
- **FANCD1 (BRCA2)**: 13q12.3
- **FANCD2**: 3p25.3
- **FANCE**: 6p22
- **FANCF**: 11p15
- **FANCG**: 9p13
DNA damage stalls replication forks and activates a protein complex containing FANC-A, -C, -E, -F, and -G.

FANC-D2 is monoubiquitylated.

FANC-D2 forms DNA repair foci with BRCA1, FANC-D1/BRCA2, RAD51, and DNA repair enzymes.
Leukemic Transformation

• Fanconi anemia patients – predisposed to many malignancies (avg. age 16 as opposed to 68 for the general population; some of the most common solid tumors are head/neck and esophageal)

• 120 of 754 registered FA patients have developed hematologic malignancies (60 AML, 53 MDS, and 5 ALL)

Inherited Defects in Hemoglobin

A heterogeneous group of genetic disorders affecting the hemoglobin loci.

- Mutations that affect protein structure – function
  sickle cell anemia, other “hemoglobinopathies”
- Mutations that affect protein quantity – the thalassemias
Overview of Hemoglobin Structure

Each hemoglobin holds a heme ring: porphyrin molecule bound to an iron (Fe) (We will not cover porphyrias today.)

Adult hemoglobin is a tetramer of alpha and beta subunits. We will focus on alpha and beta hemoglobin mutations.
Sickle Cell Anemia

**Incidence:** ~ 1/500 African Americans

**Chromosome:** 11; inherited as a recessive condition

**Defective Gene:** Beta Globin (Hemoglobin Beta Subunit)

**Deficiency:** Under low oxygen tension, the sickle cell mutation in beta globin (HbS) polymerizes to form rigid structures within the red cell. Exacerbated in “sickle crisis” episodes.
Sickle Cell Anemia
Splenic congestion and infarct in sickle cell anemia
### α Thalassemias: A Study in Gene Dosage

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hb</th>
<th>MCV</th>
<th>HbH ($\beta_4$)</th>
<th>$\alpha:\beta$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent carrier (α thal 2)</td>
<td>14-16 g/dL</td>
<td>90 μm$^3$</td>
<td>0 %</td>
<td>0.91</td>
</tr>
<tr>
<td>Alpha thal trait (α thal 1)</td>
<td>12-13 g/dL</td>
<td>70-80 μm$^3$</td>
<td>Rare inclusions</td>
<td>0.80</td>
</tr>
<tr>
<td>HbH disease</td>
<td>6-10 g/dL</td>
<td>60-70 μm$^3$</td>
<td>5-30 %</td>
<td>0.66 - 0.28</td>
</tr>
</tbody>
</table>

**Hydrops fetalis**
Hemoglobin $\alpha$ Deletions I
Hemoglobin $\alpha$ Deletions II

MCS-R 1 2 3

(\(\alpha\alpha\))AS
(\(\alpha\alpha\))CMO
(\(\alpha\alpha\))IC
(\(\alpha\alpha\))IDF
(\(\alpha\alpha\))JM
(\(\alpha\alpha\))MB
(\(\alpha\alpha\))MM
(\(\alpha\alpha\))TAT
(\(\alpha\alpha\))Ti
(\(\alpha\alpha\))00053
(\(\alpha\alpha\))04102
(\(\alpha\alpha\))L
(\(\alpha\alpha\))SN
(\(\alpha\alpha\))IJ
(\(\alpha\alpha\))LM
(\(\alpha\alpha\))Sco
(\(\alpha\alpha\))00389
(\(\alpha\alpha\))00611
(\(\alpha\alpha\))ZW
(\(\alpha\alpha\))RA
(\(\alpha\alpha\))LMB
(\(\alpha\alpha\))

}\ { Sollaino et al, Blood 2010 }
β Thalassemias: Allelic heterogeneity
Inherited Defects in Hemostasis

A heterogeneous group of genetic disorders affecting the many loci.

- Example promoting bleeding:
  Hemophilia A mutations in Factor VIII

- Example promoting clotting:
  The Factor V Leiden Mutation
Hemophilia A

- Incidence 1:5,000 - 1:10,000 males
  - about as rare as the birth of triplets
  - ~ 1 in 5,000 live male births are affected.
  - ~ 15,000 to 20,000 people with hemophilia in the US

- Hemarthroses, post-traumatic and post-surgical bleeding

- Severity related to factor VIII level
  - <1% = severe
  - 1-5% = moderate
  - 5-15% = mild

- Inhibitors develop in ~10-20% of severe patients
Mild hemophilia (factor levels >5% and <50%)
- usually bleed only after injury or surgery
- some never have a major bleed, others have several episodes depending on functional factor levels
- carriers of hemophilia may fall in the mild range

Moderate hemophilia (factor levels 2% to 5%)
- bleed about one a month, usually after trauma, surgery, or exertion.
- once a bleeding history is established in an area, may have spontaneous bleeding episodes into those areas

Severe hemophilia (factor levels <1%)
- bleed very easily, sometimes spontaneously with no warning and for no apparent reason, usually targeting the joints but potentially in any area
Hemophilia A: Genetics

- X-linked inheritance
  - ~1/3 patients represent new mutations (Haldane hypothesis)
- Germinal mosaicism
X-Linked Recessive Inheritance

- Affected males (XY):
  - sons unaffected (no male to male transmission)
  - daughters obligate carriers
- Carrier female (XX):
  - ½ sons affected; ½ daughters carriers
- Affected females: very rare
Secondary Hemostasis
Common Pathway Inactivation

*Thrombin also binds to thrombomodulin. This complex converts protein C (again a proenzyme) into its activated (enzyme) form: APC. APC in turn inactivates factor Va (factor Vi).*
Activated Protein C (APC) cofactors

APC has two known cofactors: Protein S and Factor V.

Protein S:
   Protein S enhances binding of APC to the phospholipid of platelets and endothelial cells.

Factor V:
   Factor V together with Protein S makes APC degrade FVIIIa and FVa more effectively.
Factor V Leiden

Factor V Leiden thrombophilia is an inherited disorder of blood clotting.

Factor V Leiden is a mutated form of factor V that causes an increase in blood clotting (hypercoagulability). In this disorder, the Leiden variant (form) of factor V cannot be inactivated by activated protein C, and so clotting is encouraged.

Between 3 and 8 percent of people with European ancestry carry one copy of the factor V Leiden mutation in each cell, and about 1 in 5,000 people have two copies of the mutation.
Effects of Factor V Leiden Mutation

Diagram showing the effects of Factor V Leiden mutation on thrombin activation and APC inactivation compared to normal Factor V.
“Loss-of-Function” Mutations

Thalassemias, hemophilias. Many potential loss-of-function, partial loss-of-function mutational mechanisms. (i.e., allelic heterogeneity)

“Gain-of-Function” Mutations

Sickle cell anemia, Factor V Leiden. A relatively restricted mutational spectrum will lead to the specific acquisition of the molecular characteristic that causes disease.
Somatic mutations in tumorigenesis: Gain-of-Function mutations in NOTCH1
Somatic mutations in tumorigenesis: Loss-of-Function mutations in NOTCH1
Wrap-Up

1. You’ve seen what normal bone marrow looks like and you can recognize the cells produced by hematopoiesis.

2. You’ve been exposed to platelet aggregation, the clotting factors, and the concept that hemostasis is a balance of pro-coagulant and anti-coagulant activities.

3. You’ve seen some examples of genetic defects affecting hematopoiesis and coagulation and heard about some associated lab testing.

4. You can begin to think of mutations as “loss-of-function” and “gain-of-function” types, both in inherited diseases and in somatically acquired mutations.