Biology of Hematopoiesis

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Attending Physician
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DISCLOSURES

Off-Label Usage
• None

Interests
• Celgene
• Galena
• Incyte
• Novartis
DISCLOSURES

Off-Label Usage
  • None

Financial Relationships with Relevant Commercial Interests
  • None
Hematopoiesis

- Hematopoiesis is the orderly continuous process by which primitive hemopoietic progenitor cells give rise to the mature circulating blood cells responsible for oxygen transport, host defense and hemostasis.
Requirements of Hematopoiesis

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Life Span (days)</th>
<th>Turnover Rate (cells/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>120</td>
<td>$10^{12}$</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0.5</td>
<td>$10^{11}$</td>
</tr>
<tr>
<td>Platelets</td>
<td>9</td>
<td>$10^{11}$</td>
</tr>
</tbody>
</table>
Hematopoiesis is not merely a process but a unique organ system with specific characteristics

- Hematopoiesis has a distinct ontogeny, anatomy and physiology
- Hematopoiesis is hierarchical
- Hematopoiesis is clonal and normally polyclonal
- Hematopoiesis is both deterministic and random in behavior
## Ontogeny of Hematopoiesis

<table>
<thead>
<tr>
<th>% Body Weight</th>
<th>Site</th>
<th>Mature Cell</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>Yolk sac Intravascular Liver, spleen Extravascular (Intravascular) Appendicular Bone marrow Extravascular (Intravascular) Axial</td>
<td>Nucleated red cells</td>
<td>Embryonic</td>
</tr>
<tr>
<td>Fetus</td>
<td>1.5</td>
<td>Extravascular (Intravascular) Appendicular Bone marrow Extravascular (Intravascular) Axial</td>
<td>Enucleate Red cells</td>
</tr>
<tr>
<td>Adult</td>
<td>4.5</td>
<td>Extravascular (Intravascular) Axial</td>
<td>Enucleate Red cells</td>
</tr>
</tbody>
</table>
Normal Bone Marrow Biopsy (H&E)
The Hematopoietic Microenvironment

- Mammalian hematopoiesis is normally extravascular after birth
- Within the marrow, hematopoietic progenitor cells differ in their location according to their lineage
- Stromal cells essential for promoting hematopoiesis include: fibroblasts, osteoblasts, adipocytes, endothelial cells, reticular cells, and macrophages
Stromal elements essential for promoting hematopoiesis include: the various collagens, fibronectin, laminin and the glycosoaminoglycans.

Stromal cells synthesize soluble and membrane-bound growth factors, matrix proteins and glycosoaminoglycans that tether growth factors.

Hematopoietic progenitor cells express adhesion receptors (integrins) and homing proteins for cell-cell and cell-matrix interactions.
Mechanisms for Stem Cell Migration

Osteoblast Niche → Stem Cells → Vascular Niche

- TPO
- Mpl
- + Other chemokines and adhesive proteins

Circulation → Spleen

Marrow

Commitment & Differentiation
Expansion of Hematopoiesis with Increased Demand
Paravertebral Extramedullary Hematopoiesis
Polycythemia Vera: Extramedullary Hematopoiesis
Primary Myelofibrosis: Extramedullary Hematopoiesis
Leukoerythroblastic Reaction
Causes of Extramedullary Hematopoiesis and Leukoerythroblastic Reactions

- Carcinoma metastatic to the bone marrow
  - (prostate, breast, lung, stomach)
- Lymphoma involving the bone marrow
  - (Hairy cell leukemia, CLL)
- Primary myelofibrosis
- Polycythemia vera
- Chronic myelogenous leukemia
- Myelodysplasia
- Acute hepatic injury
- Chronic hemolysis
- Recombinant hematopoietic growth factor therapy
Hematopoietic Growth Factors

- Hematopoietic growth factors (except for erythropoietin) exhibit redundancy, pleiotrophy, and synergy
  - Growth factor production is redundant since stromal cells can synthesize more than one type of growth factor
  - Each has multiple functions and stimulates more than one type of progenitor cell
  - Most have overlapping functions
  - Combinations of growth factors are more effective than individual ones
Growth factor synthesis is highly localized with growth factor tethering

Myeloid growth factors influence both primitive progenitor cells and their mature progeny

Growth factors act to:
- Maintain target cell viability
- Initiate cell cycle activity
- Activate effector functions
<table>
<thead>
<tr>
<th>Factor</th>
<th>Source</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte-macrophage colony stimulating factor</td>
<td>T lymphocytes, Mesenchymal cells</td>
<td>Stimulates macrophage and granulocyte proliferation and activation and the proliferation of other hematopoietic progenitor cells</td>
</tr>
<tr>
<td>Granulocyte colony Stimulating factor</td>
<td>Monocytes, Mesenchymal cells Neutrophils</td>
<td>Stimulates granulocyte progenitor cell proliferation and activation and costimulates other hematopoietic progenitor cell proliferation</td>
</tr>
<tr>
<td>Macrophage colony Stimulating factor</td>
<td>Mesenchymal cells Monocytes</td>
<td>Stimulates macrophage proliferation and differentiation</td>
</tr>
<tr>
<td>Factor</td>
<td>Source</td>
<td>Function</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Kidney, Liver</td>
<td>Stimulates erythroid progenitor cell proliferation</td>
</tr>
<tr>
<td>Stem Cell Factor</td>
<td>Marrow Stromal Cells</td>
<td>A costiumulator of both primitive and committed hematopoietic progenitor cells and erythroid cells</td>
</tr>
<tr>
<td>Thrombopoietin</td>
<td>Liver, Kidney</td>
<td>Stimulates megakaryocytopoiesis and thrombopoiesis</td>
</tr>
<tr>
<td>Leukemia Inhibitory Factor</td>
<td>T lymphocytes and many other types of cells</td>
<td>Stimulates proliferation of some cell types, causes differentiation of leukemic cells, inhibits embryonic stem cell differentiation</td>
</tr>
<tr>
<td>FL Ligand</td>
<td>Ubiquitous</td>
<td>A costiumulator of multipotent hematopoietic and B lymphoid progenitor cells and myeloid cells</td>
</tr>
</tbody>
</table>
Essential Factors in Erythropoiesis

- Intensity of the stimulus for red cell production
- Functional capacity of the bone marrow
- Available nutrients
- Red cell survival
Hypoxic Regulation of Erythropoietin Production

Decreased oxygen delivery to the kidneys

Cortical peritubular interstitial cells produce and secrete EPO into the blood

Erythropoiesis is increased in the marrow

Increased oxygen delivery to tissues decreases EPO production

More reticulocytes enter circulating blood

Larger number of RBCs in circulation

Confidence Intervals for Serum Immunoreactive Erythropoietin

S.E. = 324.8 − 27.2 (Hgb)

r = 0.88
Bone marrow

Sinusoidal wall

Circulation

**Bone marrow**

**Sinusoidal wall**

**Circulation**

**BFU-E**

**CFU-E**

**Pronormoblast**

**Basophilic normoblast**

**Polychromatic normoblast**

**Orthochromatic normoblast**

**Reticulocyte**

**Erythrocyte**

**EPOR +**

**Fas/TRAIL-R/TNF-R, IFNγ**

**EPOR -**

**Fas/FasL/TRAIL/TNFα**

**Caspase-3**

**Erythropoiesis**
The Major Functions of EPO are Reflected in Its Plasma Level

<table>
<thead>
<tr>
<th></th>
<th>Production</th>
<th>Plasma Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid cell viability factor</td>
<td>Constitutive</td>
<td>Constant</td>
</tr>
<tr>
<td>Erythroid cell mitogen</td>
<td>Inducible</td>
<td>Variable</td>
</tr>
</tbody>
</table>
Serum Immunoreactive Erythropoietin in Iron Deficiency Anemia

![Graph showing serum immunoreactive erythropoietin (sEPO) levels in different Hgb (g/dL) ranges with significance level P < .005.](image-url)
Association Between Hemoglobin and Serum Erythropoietin Levels in Various Disease States

A – Hemolysis
B – Iron Deficiency
C – Anemia
D – Diabetes
E – Cancer
F – Inflammation
G – Infection
H – Alcoholism
Loss of the Hgb-Serum EPO Correlation When the Serum Creatinine is Greater than 1.5 mg %
<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematologic Response</td>
<td>3.43</td>
<td>(3.07 - 3.84)</td>
</tr>
<tr>
<td>Reduction in transfusions</td>
<td>0.64</td>
<td>(0.60 – 0.68)</td>
</tr>
<tr>
<td>Risk of Thromboembolism</td>
<td>1.58</td>
<td>(0.94 - 2.66 )</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>0.81</td>
<td>(0.67 – 0.99)</td>
</tr>
<tr>
<td><strong>2007</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk of Thromboembolism</td>
<td>1.67</td>
<td>(1.35 - 2.06 )</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>1.08</td>
<td>(0.59 – 1.18)</td>
</tr>
</tbody>
</table>
## Analyses of Recombinant Erythropoietin Therapy in Cancer Patients

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Patient Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA Approval (1993)</td>
<td>413 (3 studies)</td>
</tr>
<tr>
<td>ASH/ASCO Meta-analysis (2002)</td>
<td>1,927 (22 studies)</td>
</tr>
<tr>
<td>Cochrane Meta-analysis (2005)</td>
<td>3,287 (27 studies)</td>
</tr>
<tr>
<td>Cochrane Meta-analysis (2007)</td>
<td>9,353 (57 studies)</td>
</tr>
<tr>
<td>ASH/ASCO Meta-analysis (2007)</td>
<td>11,757 (59 studies)</td>
</tr>
<tr>
<td>Cochrane Meta-analysis (2008)</td>
<td>13,933 (53 studies, ITT)</td>
</tr>
</tbody>
</table>

- **On Study Mortality**: 1.10 (0.98 – 1.24)
- **Overall Survival**: 1.04 (0.97 – 1.11)
Pluripotent Hematopoietic Stem Cell

- T Lymphocytes
- B Lymphocytes
- Granulocyte-Monocyte Progenitors
- Erythroid Progenitors
- Megakaryocytic Progenitors

Common Hematopoietic Stem Cell
The Hematopoietic Stem Cell Disease Hierarchy

- CD34+ Lin-, ALDH+, Dr^low, ABC/MDR^+
- CD34+ Lin-, ALDH+, DR^+
- CD34+ Lin+, ALDH^low, DR^+
- Lin^+

*Likely origin of CML, MPD, MDS and some AL

1. **Autoimmune Disorders**
   - Marrow and circulating blood cells
2. **ALL**
3. **AA, PNH**
4. **Primitive Stem Cells**
   - “Low Quality Stem Cells”

*Likely origin of CML, MPD, MDS and some AL
Most Acute Leukemias arise in a Pluripotent Stem Cell
Evolution of a Clonal Hematopoietic Tumor with Clonal Dominance

Normal Stem Cells

Neoplastic Stem Cells

TRANFORMATION
Clinical Analysis of Clonality in Hematology

- **Cytogenetics** (conventional, FISH)
- **Gene mutations**
  - BCR-ABL; JACK2 V617F/JAK2 exon 12; MPL W515K/L; F1P1L1-PDGFRalpha
- **Gene rearrangements**
  - Immunoglobulin genes
  - T cell receptor gene
  - BCL2 gene

- **X chromosome-linked polymorphisms**
- **Gene product analysis**
  - Immunofixation electrophoresis
  - Surface markers (flow cytometry, immunohistochemistry)
  - Isoenzymes (X-linked polymorphisms*)

*These assays are imprecise*
Analysis of Clonality in Polycythemia Vera using G-6-PD Isoenzyme Analysis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% G-6-PD A</th>
<th>%G-6-PD B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Adapted from N Engl J Med 295:913, 1976
Stem Cell Disorders Associated with Increased Blood Production

WHO classification of myeloid neoplasms and acute leukemia

- **Myeloproliferative Neoplasms (MPN)**
  - Chronic myelogenous leukemia, $BCR-ABL1$-positive
  - Chronic neutrophilic leukemia
  - Polycythemia vera
  - Primary myelofibrosis
  - Essential thrombocythemia
  - Chronic eosinophilic leukemia, not otherwise specified
  - Mastocytosis
  - Myeloproliferative neoplasms, unclassifiable

Blood 114:937,2009
Stem Cell Disorders Associated with Increased Blood Production (Continued)

- Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of PDGFR, PDGFRB, or FGFR1
  - Myeloid and lymphoid neoplasms associated with PDGFR rearrangement
  - Myeloid neoplasms associated with PDGFRB rearrangement
  - Myeloid and lymphoid neoplasms associated with FGFR1 abnormalities

Blood 114:937,2009
Stem Cell Disorders Associated with Increased Blood Production (Continued)

- Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
  - Chronic myelomonocytic leukemia
  - Atypical chronic myeloid leukemia, *BCR-ABL1*-negative
  - Juvenile myelomonocytic leukemia
  - Myelodysplastic/myeloproliferative neoplasm, unclassifiable
    - Provisional entity, refractory anemia with ring sideroblasts and thrombocytosis
The chronic myeloproliferative neoplasms are clonal disorders involving a multipotent hematopoietic progenitor cell, in which there is overproduction of one or more of the formed elements of the blood in the absence of a definable stimulus, extramedullary hematopoiesis and transformation to myelofibrosis or acute leukemia at variable but low rates.
12 Reasons Why PV, PMF, and ET Deserve a Classification Separate from the Other Myeloproliferative Disorders

1. These three disorders share in common mutations in JAK2 and MPL
2. Constitutive signal transduction in these disorders occurs through normal signaling pathways
3. These disorders cluster within families
4. First degree relatives of an afflicted proband have a 3-7 fold increased incidence of acquiring of any of the three disorders
5. These three disorders share cytogenetic abnormalities of chromosomes 1, 8, 9, 13 and 20
6. These three disorders have a high frequency of mitotic recombination of chromosome 9.
12 Reasons Why PV, PMF, and ET Deserve a Classification Separate from the Other Myeloproliferative Disorders (Continued)

7. Even with supportive therapy alone, survival is usually measured in decades
8. In some ET patients, hematopoiesis is polyclonal
9. In most true ET patients, life span is normal
10. Phenotypic mimicry occurs between these disorders but not between them and the other “MPN”
11. These disorders are diseases of myeloaccumulation, not myeloproliferation
12. The JAK2 mutations characteristic of these disorders are associated with a specific germline JAK2 gene haplotype
The Interrelationships Between the Chronic Myeloproliferative Disorders

Primary Myelofibrosis

Isolated Thrombocytosis → Polycythemia Vera

“Essential Thrombocytosis”

“All Pathways lead to polycythemia vera”
Polycythemia vera is the ultimate consequence of the JAK2 V617F mutation.
Features “Unique” to Specific “Chronic Myeloproliferative Disorders”

<table>
<thead>
<tr>
<th>Condition</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia vera</td>
<td>Erythrocytosis</td>
</tr>
<tr>
<td>Idiopathic Myelofibrosis</td>
<td>Elevated circulating CD34+ cells (early only)</td>
</tr>
<tr>
<td>“Essential Thrombocytosis”</td>
<td>None</td>
</tr>
</tbody>
</table>
Causes of Absolute Erythrocytosis

- **Hypoxia**
  - Carbon monoxide intoxication (tobacco abuse, environmental)
  - High affinity hemoglobins
  - High altitude
  - Pulmonary disease
  - Right to left shunts
  - Sleep apnea

- **Renal Disease**
  - Renal artery stenosis
  - Focal sclerosing or membranous glomerulonephritis
  - Renal transplantation

*Only ~5% of erythrocytosis patients are likely to have polycythemia vera*
Causes of Absolute Erythrocytosis (Continued)

- **Tumors**
  - Hypernephroma
  - Hepatoma
  - Cerebellar hemangioblastoma
  - Uterine fibromyoma
  - Adrenal tumors
  - Meningioma
  - Pheochromocytoma

- **Drugs**
  - Androgenic steroids

- **Familial**
  - (with normal hemoglobin function; Chuvash; EPO receptor mutations; 2, 3 BPG deficiency)

- **Polycythemia vera**
- **JAK2 V617F**
- **JAK2 exon 12 mutations**

*Only ~5% of erythrocytosis patients are likely to have polycythemia vera*
Causes of Relative Erythrocytosis

- **Loss of Fluid from the Vascular Space**
  - Emesis, diarrhea, diuretics, sweating, polyuria, hypodipsia, hypoalbuminemia, capillary leak syndromes, burns, peritonitis

- **Chronic Plasma Volume Contraction**
  - Hypoxia from any cause
  - Androgen therapy
  - Recombinant erythropoietin therapy
  - Hypertension
  - Tobacco use
  - Pheochromocytoma
  - Ethanol abuse
  - Sleep apnea
Causes of Thrombocytosis

- Tissue Inflammation
  - Collagen vascular disease, inflammatory bowel disease
- Malignancy
- Infection

- Myeloproliferative Disorders
  - Polycythemia vera, idiopathic myelofibrosis, essential thrombocytosis, chronic myelogenous leukemia

- Myelodysplastic Disorders
  - 5q-syndrome, idiopathic refractory sideroblastic anemia
Causes of Thrombocytosis (Continued)

- Postsplenectomy or hyposplenism
- Hemorrhage
- Iron deficiency anemia
- Surgery
- Rebound
  - Correction of vitamin B12 or folate deficiency, post ethanol abuse
- Hemolysis
- Familial
  - Thrombopoietin overproduction, constitutive Mpl activation, K39N
Classification of myeloproliferative disorders in the JAK2 era: is there a role for red cell mass?

Laguilier, V. De Beco, B. Cassinat, S. Burcheri, P. Weinmann, P. Fenaux, J.J. Kiladjian (Bobigny, France)

In this series of unselected consecutive patients with isolated thrombocytosis referred for RCM determination, we found that 46.5% of cases would have been misdiagnosed as ET instead of PV in the absence of RCM measurement, this proportion reaching 64.5% in the group of JAKV617F patients. Those results suggest that RCM should be performed in JAK2V617F patients with isolated thrombocytosis, for proper MPD classification and management.

Leukemia 22:452, 2008
Causes of Myelofibrosis

**Malignant**
- Acute Leukemia
  - lymphocytic, myelogenous, megakaryocytic
- Chronic Myelogenous Leukemia
- Hairy Cell Leukemia
- Hodgkin’s Disease
- **Idiopathic Myelofibrosis**
- Lymphoma
- Multiple Myeloma
- Myelodysplasia
- Metastatic carcinoma
- Polycythemia Vera
- Systemic Mastocytosis

**Non Malignant**
- HIV infection
- Hyperparathyroidism
- Renal osteodystrophy
- Systemic Lupus Erythematosus
- Tuberculosis
- Vitamin D deficiency
- Thorium Dioxide exposure
- Gray Platelet Syndrome Drugs
  - (thrombopoietin analogues)
Differential Diagnosis of Primary Myelofibrosis

- Chronic myelogenous leukemia
- Polycythemia vera
- Acute myelofibrosis
- Myelodysplasia
- Hairy cell leukemia
- Primary bone marrow lymphoma
- Systemic mastocytosis
- Metastatic carcinoma
Causes of Leukocytosis

- Infection
- Inflammation
- Chronic myeloproliferative disorders (clonal)
  - Chronic myelogenous leukemia
  - Polycythemia vera
  - Primary myelofibrosis
  - Hypereosinophilia
  - Myelodysplasia
- CMMoL
- Acute leukemias (clonal)
Causes of Leukocytosis (Continued)

- Drugs
  - Corticosteroids
  - Lithium
  - G-CSF, GM-CSF
- Tobacco
- Obesity
- Exercise/Seizures
- Postsplenectomy/hyposplenism
- Rebound from myelosuppression
- Sweet’s syndrome
- Heat stroke
- Artifact
  - Cryoproteins
The Hematopoietic Stem Cell Disease Hierarchy

CD34+ Lin-, ALDH+, Dr^low, ABC/MDR^+

CD34+ Lin-, ALDH^+, DR^+

CD34+ Lin^+, ALDH^low, DR^+

Lin^+

* Likely origin of CML, MPD, MDS and some AL

Primitive Stem Cells

“Low Quality Stem Cells”

Committed Progenitor Cells

Marrow and circulating blood cells

Autoimmune Disorders

ALL

AA, PNH

*Likely origin of CML, MPD, MDS and some AL
Pluripotent Hematopoietic Stem Cell

Common Hematopoietic Stem Cell

- Granulocyte-Monocyte Progenitors
- Erythroid Progenitors
- Megakaryocytic Progenitors

Bone Marrow Cell Pools

- Pluripotent Hematopoietic Stem Cells
- Committed Hematopoietic Progenitor Cells
- Myeloblasts
- Promyelocytes
- Erythroblasts
- Proerythrocytes
- Metamyelocytes
- Bands
- Segmented Neutrophils
- Normoblasts
- Reticulocytes

- Stem Cell Pool
- Mitotic Pool
- Reserve Pool
Severe Aplastic Anemia: Marrow Aspirate and Biopsy
Diseases Causing Bone Marrow Aplasia or Hypoplasia

Inherited

- Fanconi Anemia
- Schachman-Diamond syndrome
- Dyskeratosis Congenita
- Amegakaryocytic thrombocytopenia
Diseases Causing Bone Marrow Aplasia or Hypoplasia (Continued)

**Acquired**
- Idiopathic Aplastic Anemia*
- Drug-induced Aplastic Anemia
- Direct toxicity or idiosyncratic reaction
- Myelodysplasia*
- Paroxysmal Nocturnal Hemoglobinuria*
- Large granular lymphocyte syndrome (neutropenia, red cell aplasia, thrombocytopenia, aplastic anemia)
- Thymoma (red cell aplasia, aplastic anemia)
- Pregnancy (red cell aplasia, aplastic anemia)
- Thiopurine S-Methyltransferase deficiency (pancytopenia)

*Acquired clonal disorders
Biology of DNA Repair by the Fanconi Complex
Telomeres and Telomere Length in Hematopoiesis
Biology of Telomere Repair

Telomere

DNA-protein complex

TIN2
Ku
RAP1
NBS-MRE11
RAD50
POT1

DNA sequence:
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG
AATCCC AATCCC AATCCC AATCCC

RNA template:
CAAUUCCCAAAUC

Telomerase

TERC

DKC1

Chromosome

Hematology 2007:509
# Inherited Marrow Failure Syndromes in Adults

<table>
<thead>
<tr>
<th>Fanconi Anemia</th>
<th>Dyskeratosis Congenita</th>
<th>Diamond-Blackfan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancytopenia</td>
<td>Pancytopenia</td>
<td>Anemia</td>
</tr>
<tr>
<td>Aplastic Anemia</td>
<td>Aplastic Anemia</td>
<td>-</td>
</tr>
<tr>
<td>Leukemia/MDS</td>
<td>Leukemia/MDS</td>
<td>Leukemia/MDS</td>
</tr>
<tr>
<td>Cancer (HN, Gyn, Brain)</td>
<td>Cancer (HN)</td>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Café au Lait spots</td>
<td>Pigmentation, Gray hair Oral leukoplakia</td>
<td>-</td>
</tr>
<tr>
<td>Skeletal abnormalities</td>
<td>Nail dysplasia Pulmonary fibrosis</td>
<td>Short neck</td>
</tr>
<tr>
<td>FANC gene mutations</td>
<td>Telomerase gene mutations Dyskerin gene mutations</td>
<td>RP S17, 19, 24, loss</td>
</tr>
</tbody>
</table>
Proposed Evolution of Clonal Disorders (AL, MDS, PNH) from Acquired Aplastic Anemia
Thiopurine s-Methyltransferase (TMPT) Metabolism of Azathioprine and 6-Mercaptopurine

- Azathioprine → Mercaptopurine
- Mercaptopurine → Methylmercaptopurine
- Methylmercaptopurine → 6-Thioguanine nucleotides
- Mercaptopurine → 6-Thiouric acid

Pathways:
- Azathioprine is converted to mercaptopurine by xanthine oxidase.
- Mercaptopurine is converted to methylmercaptopurine by TPMT.
- Methylmercaptopurine is converted to 6-thioguanine nucleotides by phosphoribosyltransferase.
- Mercaptopurine is converted to 6-thiouric acid by xanthine oxidase.
Pluripotent Hematopoietic Stem Cell

- T Lymphocytes
- B Lymphocytes

Common Hematopoietic Stem Cell

- Granulocyte-Monocyte Progenitors
- Erythroid Progenitors
- Megakaryocytic Progenitors
Conditions Causing Single Lineage Bone Marrow Aplasia

- Pure Red Cell Aplasia or Hypoplasia
  - Congenital
    - Diamond Black-Fan Syndrome*
  - Acquired
    - Autoimmune
    - Thymoma, T-cell mediated (LGL)
    - Drug-induced
    - Solid tumors
    - Hematological malignancies*
      - (Myelodysplasia, CML lymphoma)
    - Infection (Parovirus B19)
    - Collagen-vascular disease
    - Pregnancy
    - Drugs
    - Erythropoietin antibodies

*Clonal disorders
Conditions Causing Single Lineage Bone Marrow Aplasia (Continued)

- Pure White Cell Aplasia
  - Congenital (Kostmann’s syndrome)*
  - Autoimmune, T-cell mediated (LGL)
  - Drugs

- Pure Megakaryocytic Aplasia
  - Congenital*
  - Thymoma, T-cell mediated (LCL)
  - Autoimmune
  - Hematological Malignancies*

*Clonal disorders
# Stem Cell Defects Causing Monocytopenias

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical Phenotype</th>
<th>Genetic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamond-Blackfan syndrome</td>
<td>Red Cell Hypoplasia</td>
<td>RP mutations (S17; S19; S24)</td>
</tr>
<tr>
<td>Kostmann’s syndrome</td>
<td>Neutropenia (Acute Leukemia)</td>
<td>? G-CSFR mutations</td>
</tr>
<tr>
<td>Congenital Amegakaryocytic thrombocytopenia</td>
<td>Thrombocytopenia (Pancytopenia)</td>
<td>MPL mutations</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>Red Cell Aplasia; 5q, Aplastic Anemia Thrombocytopenia</td>
<td>RP mutation (S14)</td>
</tr>
</tbody>
</table>
# A 42-Year-Old Man

<table>
<thead>
<tr>
<th>Month</th>
<th>Events</th>
</tr>
</thead>
</table>
| July  | • Resection of brain tumor  
         • Postoperative seizures  
         • Dilantin  
         • Phenobarbital Carbamazepine  
         • Steroids  
         • 4 blood transfusions  
         • HCT 42 |
| Aug   | • Abnormal liver function tests  
         • Dilantin  
         • Carbamazepine  
         • Steroids discontinued  
         • HCT 37 |
| Sept  | • Diffuse skin rash  
         • Abnormal liver function tests  
         • Fever  
         • Phenobarbital discontinued  
         • HCT 26  
         • WBC 11,500  
         • Eos 31%  
         • PL 634,000  
         • Retic 0.2% |
≤ 0.2 ml HGF in culture dish

~1.0 ml

Single cell suspension of hemopoietic cells in semi-solid culture medium

Incubation, 37°, 7 - 10 days

+ HGF colonies
- HGF no colonies

Colony number vs. Cell number

Colony number vs. HGF
Erythroid Colony Grown In Vitro
Erythroid Colony Growth in PRCA

<table>
<thead>
<tr>
<th>Category</th>
<th>Colony Growth</th>
<th>Response to Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C.R.</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>70%</td>
</tr>
<tr>
<td>II</td>
<td>Reduced</td>
<td>25%</td>
</tr>
<tr>
<td>III</td>
<td>Undetectable</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Blood 64:71, 1984
Classification of Red Cell Aplasia or Hypoplasia

Congenital
- Diamond-Blackfan syndrome

Acquired
- Idiopathic
- Secondary
  - Hematologic Malignancies
    - (AL, MDS, CLL, NHL, HD, AILD, CML, IMF)
  - Solid Tumors
    - (Thymoma, Lung, Stomach, Breast)

- Immunologic Disorders
  - (LGL syndrome, SLE, RA, AIHA, Pregnancy, BMT, HIV, Polyglandular syndromes I and II, TEC)

- Infectious Diseases
  - (Parvovirus B19, EBV, Hepatitis A, B, C, TEC)

- Drugs
- Anti-erythropoietin antibodies
Giant Pronormoblast Seen In Parvovirus B19 Infection
Large Granular Lymphocyte
Drugs Associated with Red Cell Aplasia

<table>
<thead>
<tr>
<th>Confirmed</th>
<th>Suspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>Allopurinol</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>D-penicillamine</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Interferon alpha</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>FK506</td>
</tr>
<tr>
<td>Recombinant erythropoietin</td>
<td>Lamivudine</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
</tr>
<tr>
<td></td>
<td>Valproate</td>
</tr>
<tr>
<td></td>
<td>Sulfonamide derivatives</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
</tr>
<tr>
<td></td>
<td>Rituximab*</td>
</tr>
<tr>
<td></td>
<td>Fludarbine*</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol**</td>
</tr>
</tbody>
</table>

*probably secondary to immunosuppression leading to B19 infection
**The effect is dose-dependent
# Classification of Adult Hematopoietic Disorders

<table>
<thead>
<tr>
<th>Decreased Production</th>
<th>Clonal</th>
<th>Nonclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplastic anemia</td>
<td>Aplastic anemia</td>
<td>Aplastic anemia</td>
</tr>
<tr>
<td>Red cell aplasia</td>
<td>Red cell aplasia</td>
<td>Red cell aplasia</td>
</tr>
<tr>
<td>MDS</td>
<td>MDS</td>
<td>White cell aplasia</td>
</tr>
<tr>
<td>PNH</td>
<td>PNH</td>
<td>Megakaryocytic aplasia</td>
</tr>
<tr>
<td>Sideroblastic anemia*</td>
<td>Sideroblastic anemia*</td>
<td>Anemia due to renal disease</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Increased Production</th>
<th>Clonal</th>
<th>Nonclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia Vera*</td>
<td>Polycythemia Vera*</td>
<td>2⁰ Erythrocytosis</td>
</tr>
<tr>
<td>Essential thrombocytosis*</td>
<td>Essential thrombocytosis*</td>
<td>2⁰ Thrombocytosis</td>
</tr>
<tr>
<td>MDS (thrombocytosis; JACK2 VV617F)</td>
<td>MDS (thrombocytosis; JACK2 VV617F)</td>
<td>Leukemoid reactions</td>
</tr>
<tr>
<td>CML</td>
<td>CML</td>
<td></td>
</tr>
<tr>
<td>CMMoL</td>
<td>CMMoL</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Increased Destruction</th>
<th>Clonal</th>
<th>Nonclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNH</td>
<td>PNH</td>
<td>Hemolytic anemias</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ITP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agranulocytosis</td>
</tr>
</tbody>
</table>

*Can be JAK2 V617F+
Summary

- Hematopoiesis is hierarchical
- Hematopoiesis is clonal but stem cell defects can mimic polyclonal disorders
- Hematopoiesis is governed by both intrinsic and extrinsic signals and thus its behavior is both nonrandom and random
- An explanation for the molecular basis of both the acute leukemias and the chronic myeloproliferative disorders will be found at the level of the pluripotent hematopoietic stem cell
- Clonal disorders of hematopoiesis are often phenotypically similar to nonclonal disorders of hematopoiesis