Hematopoiesis
Hemato – Lymphopoiesis

Hematopoiesis

Bone Marrow
Spleen

Lymphopoiesis

Thymus
Lymph nodes
Spleen
Bone marrow

Cells (erythrocytes, granulocytes, monocytes, platelets) circulate in blood and tissues

Cells (lymphocytes) circulate in lymph/tissues
Some of them in blood
Properties of Hematopoietic Stem Cell (HSCs)

- Self-renewal
- Quiescence
- High resistance to radio-chemotherapy and cytostatic drugs
- Characteristic morphology (e.g., large nucleus, euchromatin)
Hematopoietic Stem Cells

- A hematopoietic stem cell (HSC) possesses the ability for self-renewal and may differentiate into any blood cell from both the myeloid and lymphoid lineages.

- During embryogenesis HSC migrate from one to another anatomical site. This developmental journey of the HSC starts with the primitive streak/yolk sac (YS) and aorta gonado mesonephros (AGM) region and continues through the fetal liver (FL) to their final destination in the bone marrow (BM).

- HSC, during the differentiation process, give rise to more mature hematopoietic progenitor cells (HPC) that lose the ability for self renewal; however, they are able to grow colonies containing functional hematopoietic cells.
Origin of HSC

- HSC are among the first stem cells that are specified during embryogenesis from epiblast (primitive ectoderm).
Origin of HSC

- Definitive primitive pre-HSC expand in the YS, and when the developing heart tube begins to propel the first hematopoietic cells in vessels (> E8.25), primitive pre-HSC become detectable in the embryo proper and colonize the luminal surface of the aorta in the so-called aorta-gonado-mesonephros region (AGM) or para-aortic splanchnopleure (P-Sp).
Stem cells are travellers

- The colonization of BM by HSC does not terminate the developmental journey of HSC. After symmetric division one of a daughter HSC has to leave the BM niche and is released into the circulation in order to find a new niche.

- This mechanism may be responsible for maintaining homeostasis between HSC niches in different areas of BM that is distributed across various bones.
Para-aortic region

Fetal liver

Fetal spleen

Yolk sac blood island

Fetal bone marrow

Skeleton
Trafficking - Immunosurveillance by circulating Stem Cells

Bone Marrow ← Blood → Bone Marrow

Bone Marrow ← Blood → Bone Marrow

Tissues Organs

Lymph
SDF-1 gradient

CXCR4
CXCR4 - SDF-1 Axis

SDF-1

CXCR4

Adhesion

PI-3K

MEK

Jak, Tyk

Phosphatases

Fak
Paxillin
p130 CAS

AKT

MAPK p42/44

IκB

NF-κB

NF-κB

Elk-1

STAT

Biological effects
Retention in BM

Homing to BM

CXCR4

SDF-1

Developmental Migration

Retention in BM

Homing to BM

CXCR4+ HSCs
Stem Cell Niches

- **Stem cell niche** describes the microenvironment in which stem cells reside and which interacts with stem cells to regulate stem cell fate (quiescence vs. self renewal and differentiation).

- Several factors have been identified that regulate stem cell characteristics within the niche including adhesion molecules, extracellular matrix components, the oxygen tension, growth factors, cytokines, and physiochemical nature of the environment including the pH, ionic strength and metabolites.
Bone Marrow

- trabecular bone
- granulocytes
- megakaryocyte
- erythroid island

[Diagram of bone marrow with labels: CXCL12-secreting reticular cell, Osteoblast, HSC, Vein, Artery, Sinusoid, Endosteum]
Stem Cell Niches

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Bone Marrow stem cell niches
HSC DIVISIONS

SYMETRIC

ASYMETRIC
Bone Marrow Examination
Hematopoietic Growth Factors
- Growth factors for many type of cells
- KL or c-kit receptor deficiency – anemia,
- Activates early steps of hematopoiesis
- Activates mast cells
Erythropoietin

- Secreted by kidneys
- Regulated by hypoxia
- Required for red cell development
Trombopoietin

- Scented by liver, kidney and spleen
- Secretion regulated by blood platelet number
- Stimulates megakaryopoiesis and thrombopoiesis
- 20% of blood platelets may be produced in TpO deficient mice
G-CSF
Granulocyte colony stimulating factor

- Stimulates myelopopoiesis (granulocyto-monopoiesis)
- Employed to mobilize HSCs from bone marrow into peripheral blood
Progenitor Cell Assays
Clonogeneic Assays

Cytokines + Growth Factors

Colonies
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF
Colony Forming Unit of Mixed Lineages (CFU-GEEM)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF

Erythropoietin

Erythropoiesis

BFU-E
Burst Forming Unit of Erythroblasts (BFU-E)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF

Erytropoiesis

Erytropoietin

BFU-E

Trombopoietin

Megakariopoiesis

CFU-Meg
Colony Forming Unit of Megakaryocytes (CFU-Meg)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF

CFU-GM

Erytropoiesis

Erytropoietin

BFU-E

Trombopoietin

Megakariopoiesis

CFU-Meg

Mielopoiesis
Colony Forming Unit of Granulocyte-Macrophages (CFU-GM)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF

CFU-GM

Kit Ligand (KL)

Erytropoiesis

Erytropoietin

BFU-E

Trombopoietin

Megakariopoiesis

CFU-Meg

G-CSF – granulocytes
M-CSF – monocytes
IL-5 – eosinophils
IL-4 bazophils

Mielopoiesis

CFU-G
CFU-M
CFU-Eos
CFU-Baso
HSC

Kit ligand (KL), Interleukin-1 (IL-1), Interleukin-3 (IL-3), Angiopoetin-1, trombopoetin

IL-7

T lymphocytes

NK Cells

B lymphocytes

IL-7

IL-2

IL-7

IL-15

IL-7

IL-4
Stem Cell Markers
HSCs Receptors

CD34

CD133

C-KIT

CXCR4
HSCs Metabolic Markers

Stem Cells are
Hoe 33341 $^{\text{low}}$
Pyronin Y $^{\text{low}}$
Rh 123 $^{\text{dim}}$
Hematopoietic Stem Cell Phenotype
Phenotype of HSCs

- CD34$^+$ CD133$^+$ CXCR4$^+$ c-kit$^+$ Lin$^-$ CD45$^+$
- Hoechst33342$^{\text{low}}$, Rhodamin123$^{\text{low}}$ and Pyronin Y$^{\text{low}}$
HSCs Isolation Strategies
**MACS beadsy**

1. Label the cells of interest with MACS MicroBeads in a short incubation step.

2. Pass the mixture of labeled and unlabeled cells over a separation column placed in the magnetic field of a MACS separator. Collect the flow trough as the non-magnetic fraction.

3. Remove the separation column from the magnet and flush out the retained cells as the positively selected cells.
FACS sorter

[Diagram of FACS sorter with labeled parts: ultrasonic nozzle vibrator, cell suspension, sheath fluid, laser, detectors, analyzer, small groups of drops negatively charged due to detection of single fluorescent cell, drop-charging signal, small groups of drops positively charged due to detection of single nonfluorescent cell, -2000 V, +2000 V, cell collector, flask for undeflected droplets, image of a person operating the machine]
Hematopoietic Transplants
Bone Marrow Transplant
Mobilized Peripheral Blood Transplant
Umbilical Cord Blood Transplant
Homing - Mobilization

Bone Marrow Niches
Both conditioning for transplant and administration of mobilizing drugs activates complement cascade.
Stem cell homing
Both conditioning for transplant and administration of mobilizing drugs activates complement cascade

Classical Pathway

- C1q
- C2
- C4

Alternative Pathway

- Factor D
- Factor B

C3

C3a, desArgC3a

Promote homing

C5

C5b-9

Membrane Attack Complex (MAC)
Developmental Migration

Retention in BM
Homing to BM

SDF-1

CXCR4

CXCR4+ HSC

Developmental Migration

Retention in BM
Homing to BM
Primimg of SDF-1-CXCR4 axis by antimicrobial peptides

**Priming effect:**

*Increase in responsiveness to SDF-1 gradient*

SDF-1 (low dose) + priming agent (e.g., C3a, LL-37)
Priming effect of C3a and LL-37 on low doses of SDF-1
Human BM- and UCB-derived CD34+ cells primed with C3a engraft faster in immunodeficient mice

Stem cell mobilization
Stem Cell Mobilization

- Development/Organogenesis
- Physiology – circadian rhythm
- Strenous exercise
- Inflammation
- Tissue/organ injury-induced mobilization (e.g., heart infarct, stroke)
- Pharmacological mobilization (e.g., G-CSF, AMD3100) – HSPC circulating in PB increase up to 100 times.
Retention of HSCs in the BM-niches is an active process that counteracts continuous chemottractive gradient of factors present in plasma ("Gravitation field analogy").
Classical Pathway

Alternative Pathway

C1q
C2
C4

C3

C5

C5a, desArgC5a

Promote mobilization

C5b-9

Membrane Attack Complex (MAC)

Ratajczak et al. *Blood* 2004;93:2071-2078
Retention of HSCs in the BM-niches is an active process that counteracts continuous chemottractive gradient of factors present in plasma ("Gravitation field analogy").

SDF-1 – CXCR4
VCAM-1 – VLA-4 (α4β1)

AMD3100 - CXCR4 antagonist
BIO4860 - VLA-4 antagonist

MOBILIZATION
C5 activation is required for mobilization of HSCs

<table>
<thead>
<tr>
<th>Normal Mice</th>
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<table>
<thead>
<tr>
<th>C5⁻/⁻ Mice</th>
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“Ice Breaker Mechanism”

“Granulocyte”

“HSC”
Classical Pathway

Alternative Pathway

C1q → C2 → C4 → C3

Factor D → Factor B

C5

C5a, desArgC5a

Promote mobilization

C5b-9
Membrane Attack Complex (MAC)

Lytic MAC
Sublytic MAC
The lytic membrane attack complex C5b-9 (MAC) forms transmembrane channels. These channels disrupt the phospholipid bilayer of target cells, leading to cell lysis and death.

The sublytic (soluble) membrane attack complex C5b-9 (MAC) may bind to cell membranes, independent of any receptor, does not lyse cells, activates multiple signaling pathways and has wide-range effects on many cell types leading to cellular responses, such as secretion, adherence, aggregation, chemotaxis and even cell division.
Sphingosine 1 phosphate

Bone Marrow

Peripheral Blood

25 x higher concentration!
Activation of myeloid cells induces proteolytic microenvironment.

**BM niche**

**Endothelium**

**BM sinusoids**

- **Mobilization activates CC**
- **C5a**
- **MAC (C5b-C9)**

**Complement Cascade**

**Gradient S1P**

**S1P**

**RBC**

**HSPC**

**Proteases**
Mobilized Peripheral Blood Transplant