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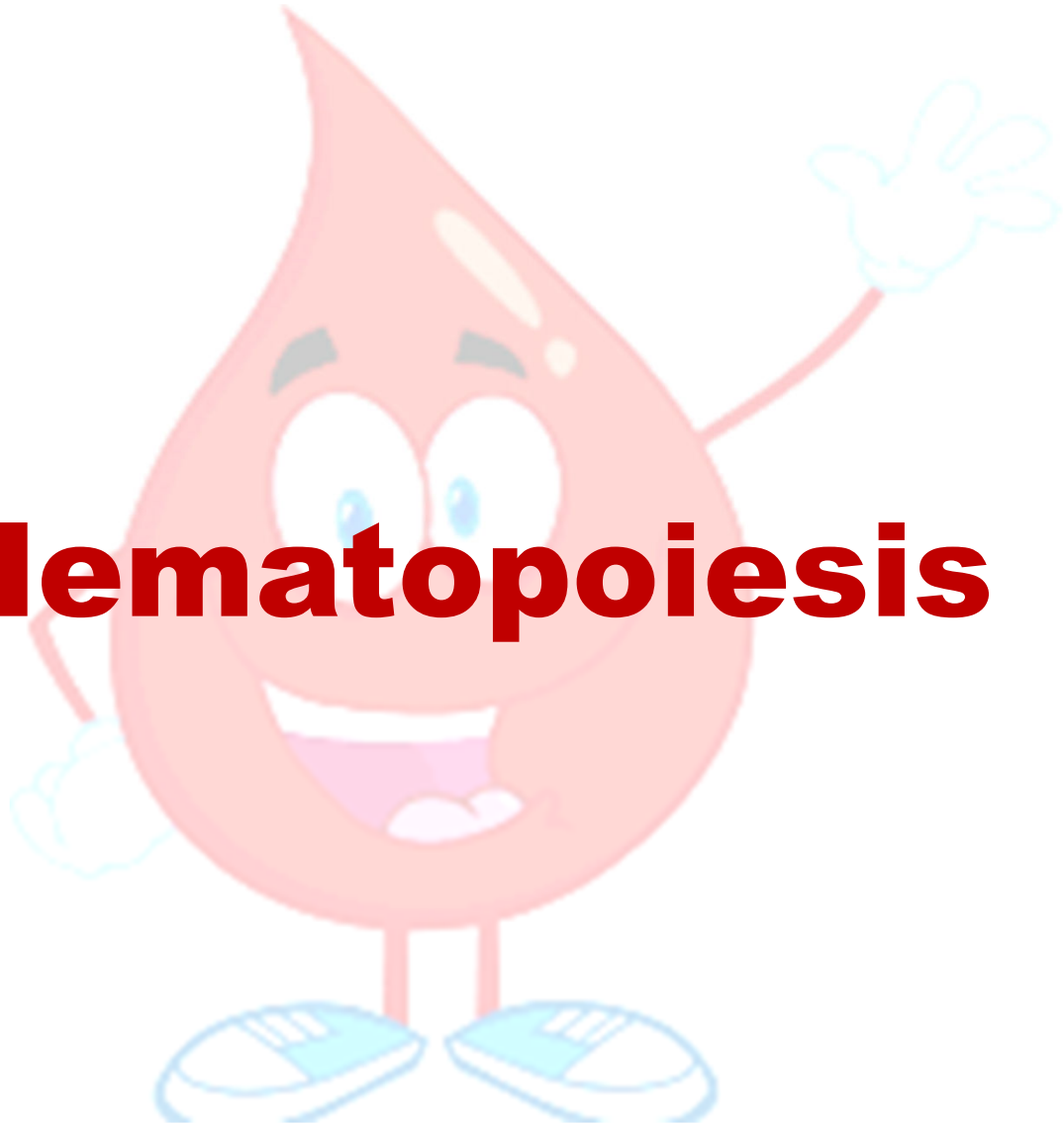

Hematopoiesis

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What is Hematopoiesis?

Hematopoiesis is the **continuous, regulated process of renewal, proliferation, differentiation, and maturation** of all **blood cell lines.**

These processes result in the **formation, development, and specialization** of all functional blood cells that are **released from the bone marrow into the circulation.**

Mature blood cells have a limited lifespan (e.g., 120 days for red blood cells [RBCs]) and a cell population capable of self-renewal that sustains the system.

A hematopoietic stem cell (HSC) is capable of self-renewal (i.e., replenishment) and directed differentiation into all required cell lineages.

Thus, the hematopoietic system serves as a functional model to study stem cell biology, proliferation, and maturation and their contribution to disease and tissue repair.

Hemangioblast and Angioblast

- The **hemangioblast,** a common precursor for hematopoietic and vascular lineages, was proposed nearly a century ago based on the close proximity of cells in the yolk sac that give rise to both blood cells and blood vessels.
- It was **Murray who in 1932 coined the term "hemangioblast"** to indicate the thickenings of the mesoderm in the chick yolk sac, the mesodermal "masses" located at the sites where later the blood islands emerge.
- **Angioblast** is one of the **extraembryonic mesenchyme cells that** differentiate into the **endothelium of the embryonic blood vessels.**
- Angioblasts form **capillary channels by vasculogenesis (de novo capillary formation) and by angiogenesis (the formation of new capillaries from existing ones).**

Site of Hematopoiesis

- Hematopoiesis in the developing human can be characterized as a select distribution of embryonic cells in specific sites that rapidly changes during development.
- In humans, hematopoiesis, the formation and development of red and white blood cells, begins in the **embryonic yolk sac during the first weeks of development.** Here, yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin. **In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the fetal liver and then to the spleen;** these two organs have major roles in hematopoiesis from the third to the seventh months of gestation. After that, the **differentiation of HSCs in the bone marrow becomes the major factor in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.**
- There are three phases. During **fetal development**, the restricted, sequential distribution of cells is initiated in the **yolk sac and then progresses in the aorta-gonad-mesonephros (AGM) region (mesoblastic phase), then to the fetal liver (hepatic phase), and finally resides in the bone marrow (medullary phase).**
- Because of the different locations and resulting microenvironmental conditions (i.e., niches) encountered, **each of these locations has distinct but related populations of cells.**

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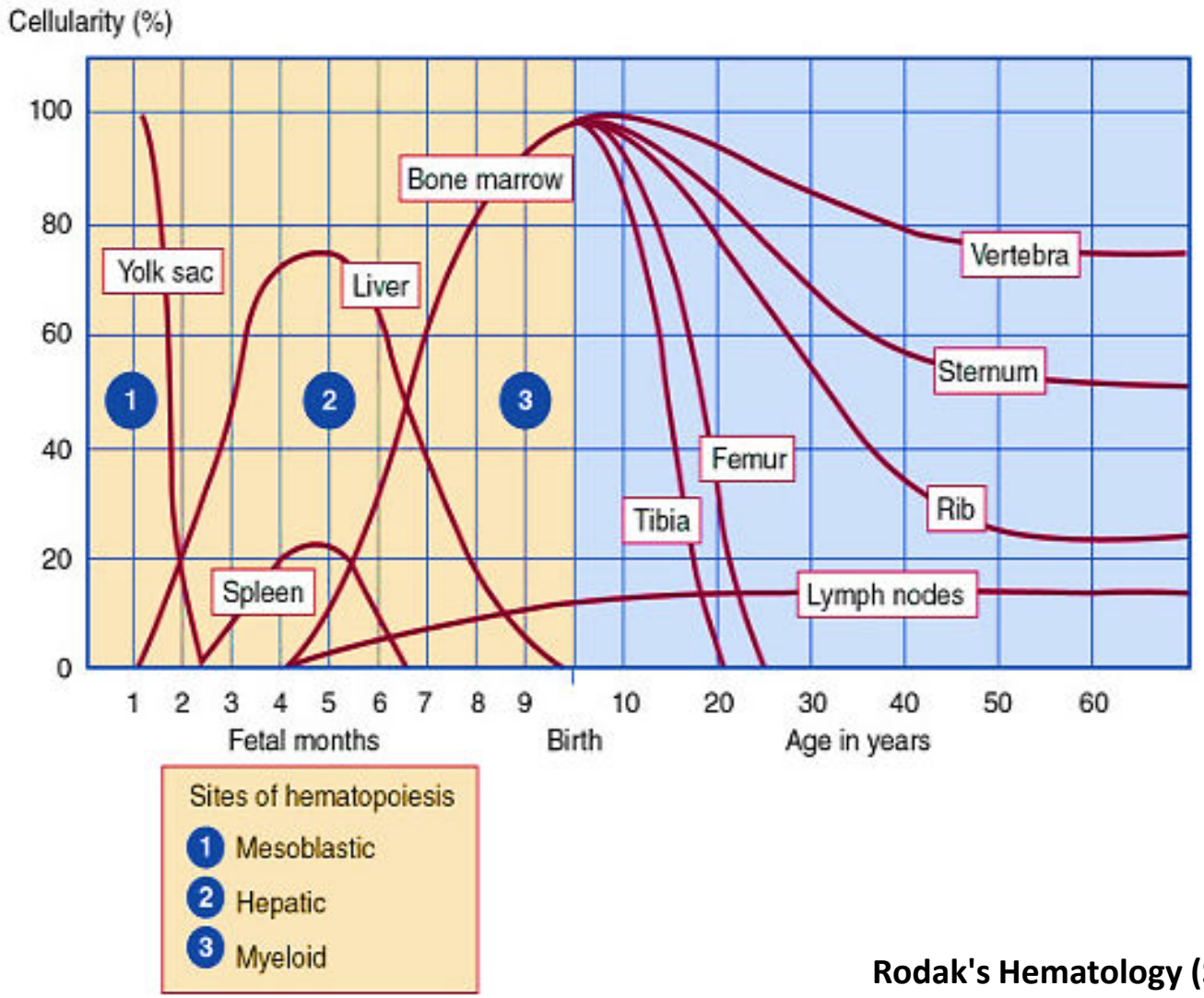

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Mesoblastic phase

- Hematopoiesis is considered to begin around the **nineteenth day of embryonic development after fertilization.**
- Early in embryonic development, **cells from the mesoderm migrate to the yolk sac. Some of these cells form primitive erythroblasts in the central cavity of the yolk sac, and others (angioblasts) surround the cavity of the yolk sac and eventually form blood vessels.**
- These primitive but transient yolk sac erythroblasts are **important in early embryogenesis to produce hemoglobin (Gower-1, Gower-2, and Portland)** needed for delivery of oxygen to rapidly developing embryonic tissues.
- Yolk sac hematopoiesis differs from hematopoiesis that occurs later in the fetus and adult in that **it occurs intravascularly (or within developing blood vessels).**
- Cells of **mesodermal origin migrate to the AGM region** and **give rise to HSCs for definitive or permanent adult hematopoiesis.**
- The AGM region was previously considered to be the only site of definitive hematopoiesis during embryonic development. However, subsequent studies clearly demonstrated that the **yolk sac was the major site of adult blood formation in the embryo.**
- Reports indicate that **Flk1+ HSCs separated from human umbilical cord blood could generate hematopoietic as well as endothelial cells in vitro.**
- Some reports indicate that **purified murine HSCs generate endothelial cells after in vivo transplantation.**
- More recently, researchers have challenged the AGM origin of HSCs based on transgenic mouse data demonstrating that **yolk sac hematopoietic cells in 7.5-day embryos express RUNX1 regulatory elements needed for definitive hematopoiesis.**
- Overall these findings suggest that the yolk sac contains either definitive HSCs or cells that can give rise to HSCs.

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Hepatic phase

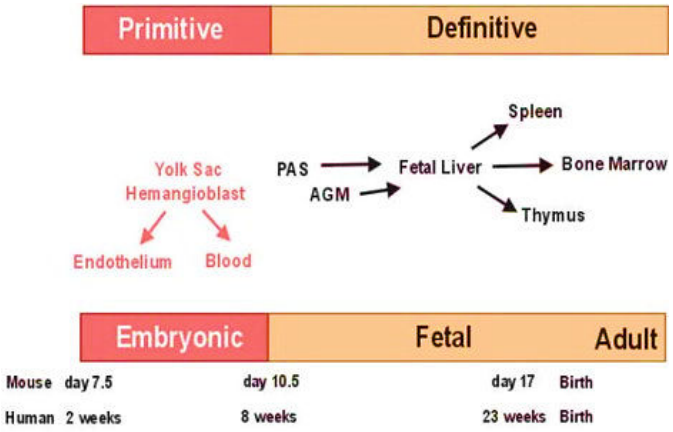
- The hepatic phase of hematopoiesis begins **at 5 to 7 gestational weeks and is characterized by recognizable clusters of developing erythroblasts, granulocytes, and monocytes colonizing the fetal liver, thymus, spleen, placenta, and ultimately the bone marrow space** in the final medullary phase.
- These varied niches support development of HSCs that migrate to them.
- Developing erythroblasts signal the beginning of definitive hematopoiesis with a **decline in primitive hematopoiesis of the yolk sac.**
- In addition, lymphoid cells begin to appear.
- Hematopoiesis during this phase **occurs extravascularly**, with the **liver remaining the major site of hematopoiesis** during the second trimester of fetal life.
- Hematopoiesis in the **AGM region and the yolk sac disappear during this stage.**
- **Hematopoiesis in the fetal liver reaches its peak by the third month of fetal development, then gradually declines after the sixth month**, retaining minimal activity until 1 to 2 weeks after birth.
- The **developing spleen, kidney, thymus, and lymph nodes contribute to the hematopoietic process during this phase.**
- The **thymus, the first fully developed organ in the fetus, becomes the major site of T cell production, whereas the kidney and spleen produce B cells.**
- **Production of megakaryocytes begins** during the hepatic phase.
- The spleen gradually decreases granulocytic production and subsequently contributes solely to lymphopoiesis.
- During the hepatic phase, **fetal hemoglobin (Hb F) is the predominant hemoglobin, but detectable levels of adult hemoglobin (Hb A) may be present.**

Medullary (myeloid) phase

- Hematopoiesis in the bone marrow (termed medullary hematopoiesis because it occurs in the **medulla or inner part of the bone cavity**) begins between the **fourth and fifth month of fetal development.**
- During the myeloid phase, **HSCs and mesenchymal cells migrate into the core of the bone.**
- **Mesenchymal cells**, a type of embryonic tissue, **differentiate** into structural elements (e.g., **stromal cells such as endothelial cells and reticular adventitial cells)** that **support developing hematopoietic elements.**
- Hematopoietic activity, especially myeloid activity, is apparent during this stage of development, and the **myeloid-to-erythroid ratio gradually approaches 3:1 to 4:1 (normal adult levels).**
- By the **end of 24 weeks' gestation, the bone marrow becomes the primary site of hematopoiesis.**
- Measurable levels of erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and hemoglobins F and A can be detected.
- In addition, cells at various stages of maturation can be seen in all blood cell lineages.

Blood development in vertebrates involves two waves of hematopoiesis: the primitive wave and the definitive wave (Galloway and Zon, 2003).

- **The primitive wave,** which involves an erythroid progenitor, **gives rise to erythrocytes and macrophages during early embryonic development.** The primary purpose of the primitive wave is to produce red blood cells that can facilitate tissue oxygenation as the embryo undergoes rapid growth.
- In mammals and avians, these **erythroid progenitor cells first appear in blood islands in the extra-embryonic yolk sac** early in development.
- The primitive wave is transitory, however, and these **erythroid progenitors are not pluripotent and do not have renewal capability.**
- **Definitive hematopoiesis,** by contrast, occurs later in development, notably at different time points in different species.
- In most organisms, there is a transient wave of definitive hematopoiesis that occurs in the blood islands and produces **progenitors called erythroid-myeloid progenitors (EMPs).**
- Definitive hematopoiesis later **involves HSCs, which are multipotent and can give rise to all blood lineages of the adult organism.**
- In vertebrates, definitive **HSCs** are born in the aorta-gonad-mesonephros (AGM) region of the developing embryo. They **migrate to the fetal liver and then to the bone marrow**, which is the location for HSCs in adults.



Hematopoiesis

- Early in hematopoiesis, a multipotent stem cell differentiates along one of two pathways, giving rise to **either a common lymphoid progenitor cell or a common myeloid progenitor cell.**
- During the development of the lymphoid and myeloid lineages, **stem cells differentiate into progenitor cells, which have lost the capacity for self-renewal and are committed to a particular cell lineage.**
- **Common lymphoid progenitor cells** give rise to **B, T, and NK (natural killer) cells and some dendritic cells.**
- **Myeloid stem cells** generate progenitors of **red blood cells (erythrocytes), many of the various white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells), and platelets.**
- When the **appropriate factors and cytokines are present**, progenitor cells **proliferate and differentiate** into the corresponding cell type, either a mature erythrocyte, a particular type of leukocyte, or a platelet-generating cell (the megakaryocyte).
- Red and white blood cells pass into bonemarrow channels, from which they enter the circulation. In bone marrow, **hematopoietic cells grow and mature on a meshwork of stromal cells, which are nonhematopoietic cells that support the growth and differentiation of hematopoietic cells.**
- Stromal cells include **fat cells, endothelial cells, fibroblasts, and macrophages.**
- Stromal cells influence the differentiation of hematopoietic stem cells by **providing a hematopoietic-inducing microenvironment (HIM)** consisting of a cellular matrix and factors that promote growth and differentiation.
- Many of these hematopoietic growth factors are soluble agents that arrive at their target cells by diffusion, others are membrane-bound molecules on the surface of stromal cells that require cell-to-cell contact between the responding cells and the stromal cells.

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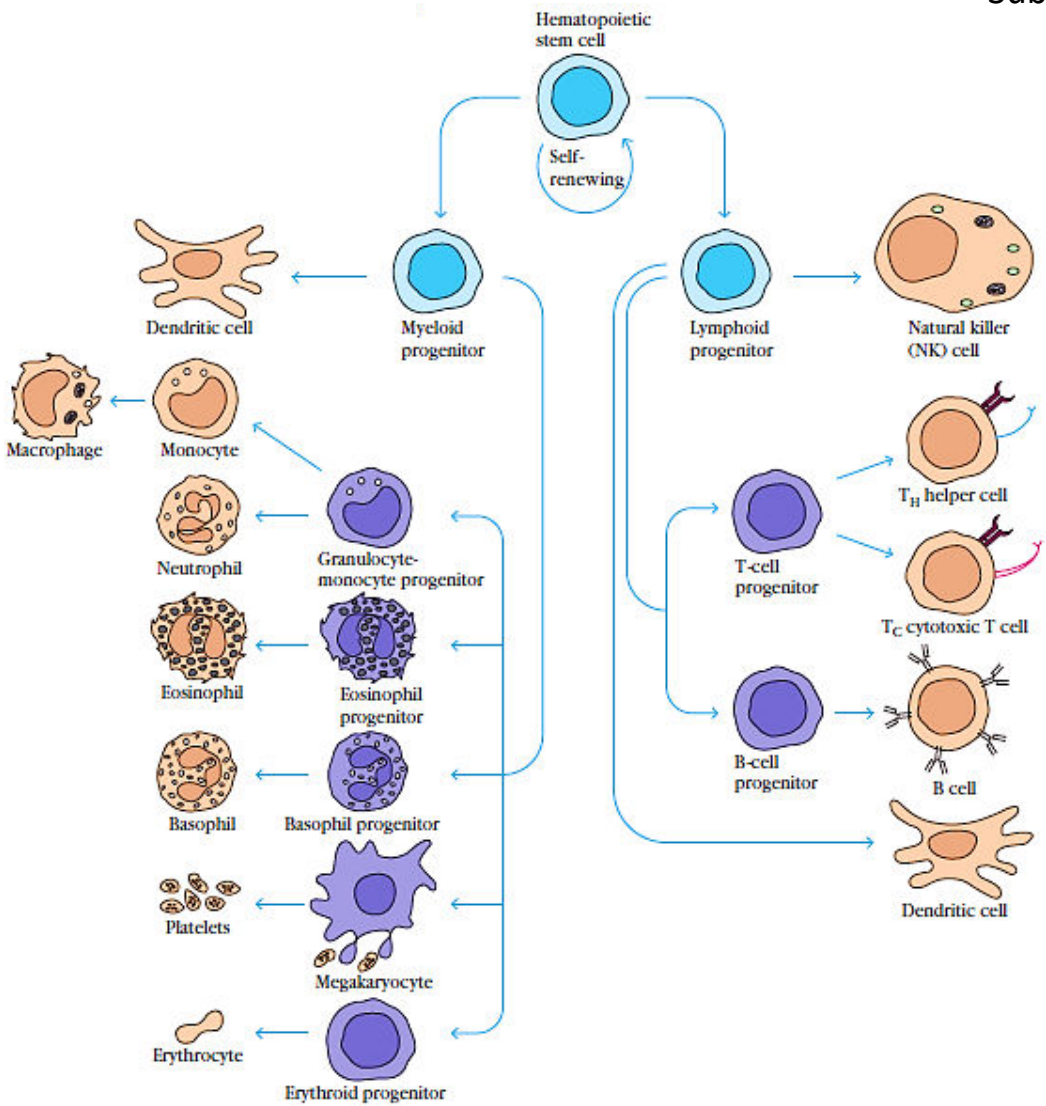
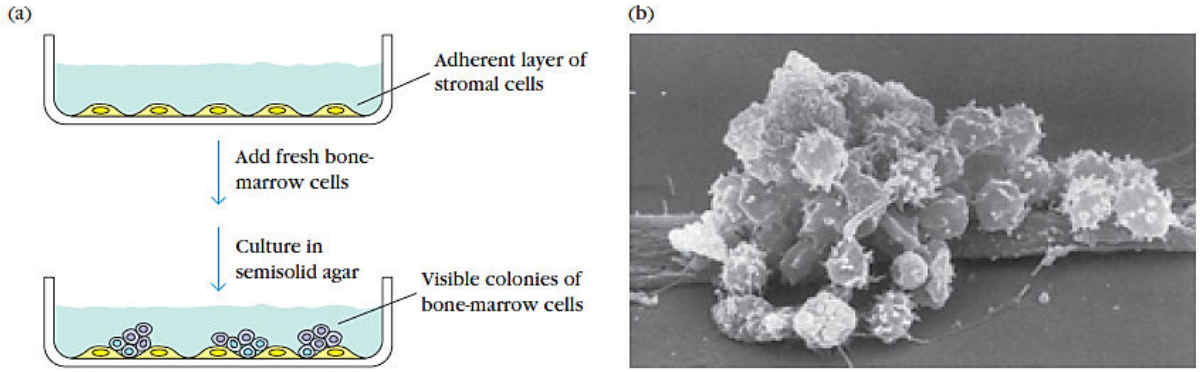
Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors



Hematopoiesis can be studied in vitro



- Bone-marrow stromal cells are cultured to form a layer of cells that adhere to a petri dish; freshly isolated bone-marrow hematopoietic cells placed on this layer will grow, divide, and produce large visible colonies.
- If the cells have been cultured in semisolid agar, their progeny will be immobilized and can be analyzed for cell types.
- Colonies that contain stem cells can be replated to produce mixed colonies that contain different cell types, including progenitor cells of different cell lineages.
- Various growth factors are required for the survival, proliferation, differentiation, and maturation of hematopoietic cells in culture.

Important cytokines

- **Hematopoietic cytokines are large family of extracellular ligands** that stimulate hematopoietic cells to differentiate into eight principle types of blood cells.
- Numerous cytokines are involved in the regulation of hematopoiesis within a complex network of **positive and negative regulators.**
- Some cytokines have very narrow lineage specificities of their actions, while many others have rather broad and overlapping specificity ranges.
- This includes **GM-CSF, G-CSF, M-CSF, interleukins, EPO and TPO.** There are a number of other cytokines that exert profound effects on the formation and maturation of hematopoietic cells, which include **stem cell factor (SCF), flt-3/flk-2 ligand (FL) and leukemia inhibitory factor (LIF).**
- Other cytokines or ligands such as **jagged-1, transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α)** also play significant roles in modulating hematopoiesis.
- **Acidic glycoproteins, the colony-stimulating factors (CSFs),** named for their ability to induce the formation of distinct hematopoietic cell lines.
- **Glycoprotein erythropoietin (EPO).** Produced by the kidney, this cytokine induces the **terminal development of erythrocytes and regulates the production of red blood cells.**

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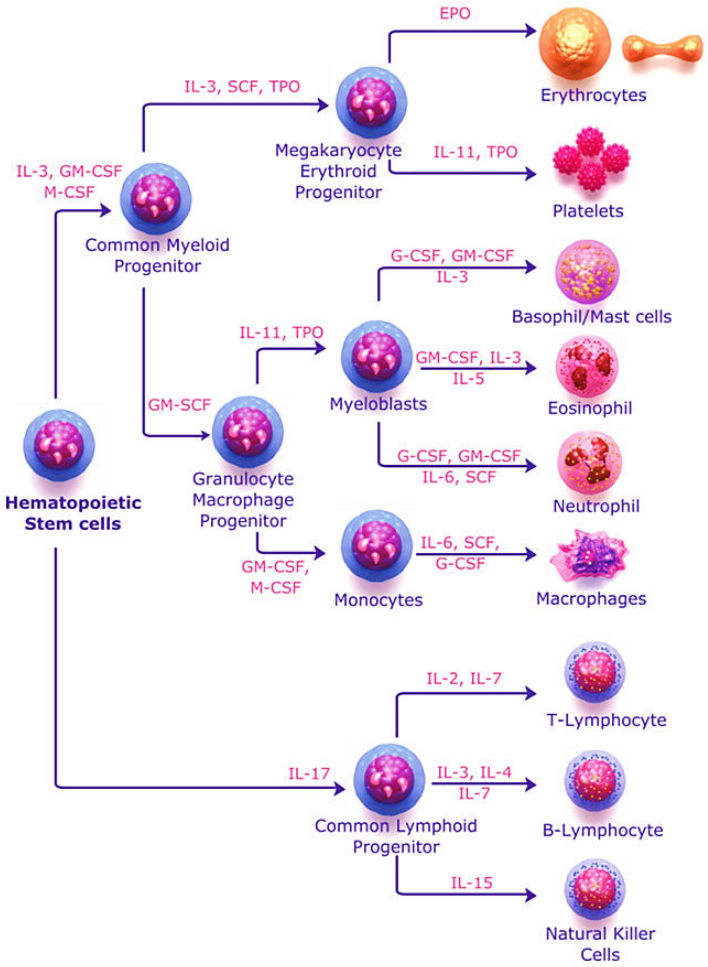
Regulation of hematopoiesis by cytokines

Cytokine	Function in hematopoiesis
Erythropoietin (EPO)	Red blood cell production
Flt-3	Stimulation of stem and developing dendritic cells
Granulocyte-macrophage colony stimulating factor (GM-CSF)	Stimulation of diverse set of granulocyte-macrophage colonies
Granulocyte-colony stimulating factor (G-CSF)	granulocytic colony stimulation
Interleukin-2 (IL-2)	T-cell proliferation
Interleukin-3 (IL-3)	Granulocyte, macrophage, eosinophil, megakaryocyte and erythroid colony formation
Interleukin-5 (IL-5)	B-cell differentiation and eosinophil regulation
Interleukin-6 (IL-6)	B-cell differentiation
Interleukin-7 (IL-7)	T-lymphocyte induction
Interleukin-11 (IL-11)	Stimulation of megakaryocytes and plasmacytoma cell lines
Leukemia inhibitory factor (LIF)	Differentiation and suppression of clonogenicity of leukemic cells
Macrophage-colony stimulating factor (M-CSF)	Macrophage colony stimulation
Stem cell factor (SCF)	Proliferation of mast cells and stem cells
Thrombopoietin (TPO)	Regulation of platelet production; stimulation of megakaryocytes with IL-3 and SCF

Hematopoietic cytokines stimulate hematopoietic cells to differentiate into principle types of blood cells



Hematopoiesis is regulated at the genetic level

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- Primitive hematopoiesis is largely regulated by two transcription factors, **Gata1 and Pu.1 (now known as Sfpi1 in mouse; Spi1b in zebrafish),** that exhibit a **cross-inhibitory relationship to regulate primitive erythroid and myeloid fates**. **Gata1 is a master regulator of erythrocyte development.**
- **Pu.1** is a master regulator of the myeloid cell fate, which includes macrophages and granulocytes.
- One transcription factor that affects multiple lineages is **GATA-2**, a member of a family of transcription factors that recognize the tetranucleotide sequence GATA, a nucleotide motif in target genes. A functional GATA-2 gene, which specifies this transcription factor, is essential for the development of the lymphoid, erythroid, and myeloid lineages.
- In contrast to GATA-2, another transcription factor, **Ikaros,** is required only for the development of cells of the lymphoid lineage.
- **Runx1** is a member of the runt family of transcription factors and plays an important role in hematopoiesis.
- In zebrafish**, Cmyb** expression begins at around the 10- to 12-somite stage during the primitive wave of hematopoiesis.

Some transcription factors essential for hematopoietic lineages

Factor	Dependent lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturational stages), myeloid (later stages), lymphoid
BM11	Myeloid, lymphoid
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

Factors regulating HSC self-renewal

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The role of Wnt signaling in HSC function

Most studies have found a positive role for Wnt in HSCs during development and regeneration. Recent findings suggest that these opposing conclusions are due to the different levels of Wnt in different experimental conditions.

The Notch signaling pathway

Activation of Notch signaling has been shown to promote HSC expansion/self-renewal in both mice and humans in adult hematopoiesis. Populations of human cells expressing CD34 (a cell surface marker for HSCs) can be expanded with exposure to Notch ligands, resulting in >100-fold increase in the absolute number of cells, which can subsequently enhance the repopulation of immunodeficient mice.

The HSC niche

The microenvironment is known to be essential for the regulation and maturation of many stem cells. The adult bone marrow niche of mice is currently the most studied HSC niche. Some studies identify the **osteoblast** as an important cell that interacts with HSCs in the bone marrow. Mutant mice with disrupted bone morphogenetic protein (BMP) signaling have increased numbers of osteoblasts and HSCs. **Vascular cells (and a vascular niche)** are also important for HSC regulation. **Stromal cells** expressing kit ligand are also required for stem cell homeostasis

Hematopoietic homeostasis involves many factors

Hematopoiesis is a continuous process that generally maintains a steady state in which the production of mature blood cells equals their loss (principally from aging). The average erythrocyte has a life span of 120 days before it is phagocytosed and digested by macrophages in the spleen. The various white blood cells have life spans ranging from a few days, for neutrophils, to as long as 20–30 years for some T lymphocytes. To maintain steady-state levels, the average human being must produce an estimated 3.7×10^{11} white blood cells per day.

Steady-state regulation of hematopoiesis is accomplished in various ways, which include:

Control of the levels and types of cytokines produced by bone-marrow stromal cells.

The production of cytokines with hematopoietic activity by other cell types, such as activated T cells and Macrophages.

The regulation of the expression of receptors for hematopoietically active cytokines in stem cells and progenitor cells.

The removal of some cells by the controlled induction of cell death.