Chapter 02: Hematopoiesis

I. Definition of Hematopoiesis:

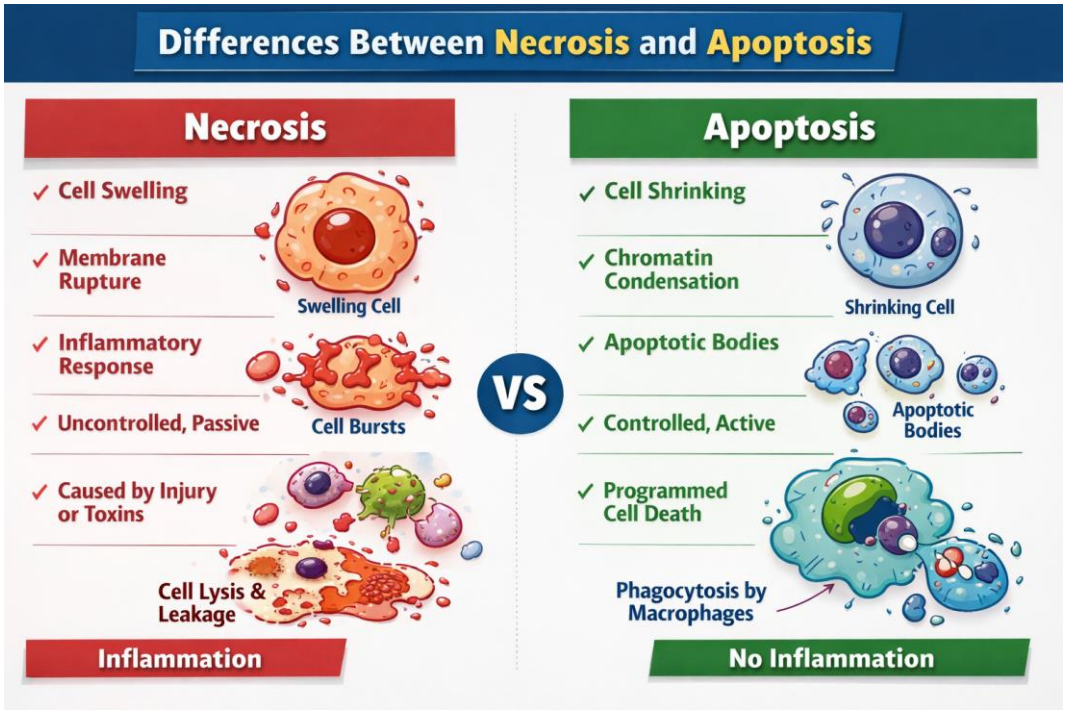
Mature functional blood cells comprise three cellular lineages: erythrocytes, leukocytes (both polymorphonuclear and mononuclear), and platelets. Despite their limited lifespan (erythrocytes: 120 days; platelets: 7 days; neutrophilic polymorphonuclear cells: 24 hours), their numbers remain constant, requiring continuous replacement through the process of hematopoiesis.

Hematopoiesis is a set of physiological mechanisms that ensure the continuous and regulated production and renewal of blood cells. It is carried out by Hematopoietic Stem Cells (HSCs) within a specific cellular microenvironment. The daily production volume is estimated at 10^{13} cells/day (RBCs: 250×10^9/day; platelets: 150×10^9/day; neutrophils: 100×10^9/day).

The study of hematopoiesis allows for the understanding of the pathophysiological mechanisms of blood disorders and the development of therapeutic strategies, including growth factors and bone marrow transplantation.

2. Programmed Cell Death

Programmed cell death, an induced and orderly process in which the cell actively participates in its own elimination, is an essential factor in the homeostatic regulation of many cell populations, including those of the hematopoietic system. Cells undergoing programmed death often exhibit characteristic morphological changes known as apoptosis. These changes include a reduction in cell volume, cytoskeletal alterations leading to plasma membrane blebbing, chromatin condensation, and DNA fragmentation into small pieces. An apoptotic cell releases tiny membrane-bound apoptotic bodies containing intact organelles. Macrophages rapidly phagocytose these apoptotic bodies, so apoptosis does not trigger a local inflammatory response.



3. Sites of Hematopoiesis:

❖ **In Humans:** The site of hematopoiesis changes throughout life.

1. **Intrauterine Life:** Three periods:
    - **Pre-hepatic period:** From day 19 to the 2nd month, at the level of the yolk sac (mesoderm).
    - **Hepatosplenic period:** From the 2nd month to the 6th month, at the level of the liver and spleen.
    - **Medullary period:** From the 6th month onward, at the level of the bone marrow (BM).
2. **After Birth:** Hematopoiesis is exclusively medullary throughout life, occurring in all bones until age 4, then restricted to short and flat bones (sternum, vertebrae, ribs, and iliac bones).

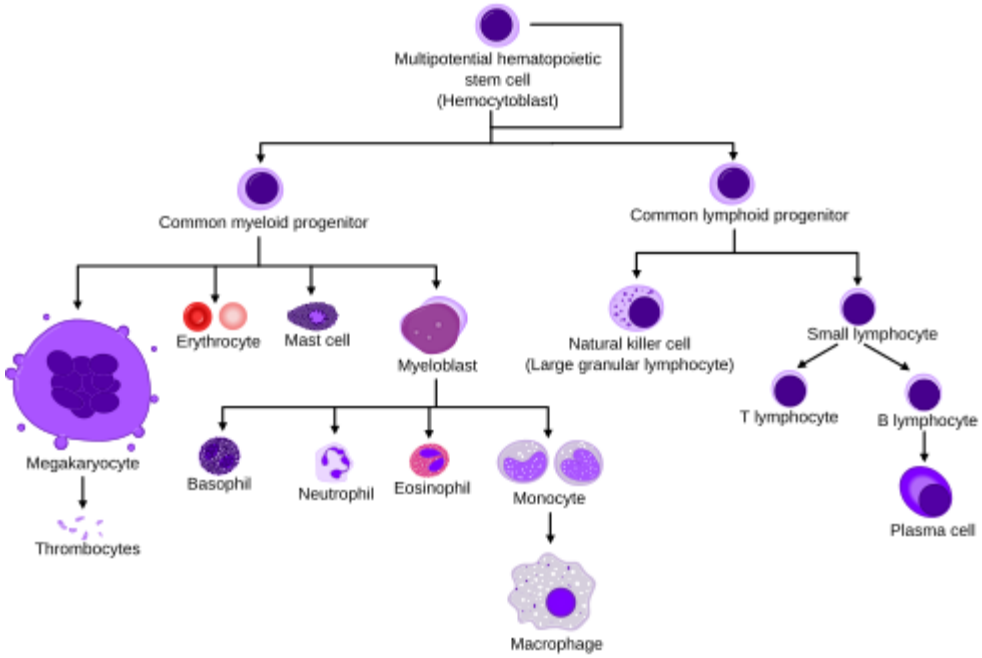
❖ **In Mice:** Hematopoiesis occurs in the bone marrow and spleen.

4. Hematopoietic Compartments

All blood cells are produced from a single undifferentiated cell, the **hematopoietic stem cell (HSC)**.

Under the influence of stimulating factors, a multipotent HSC commits to differentiation toward a specific cell lineage. The multipotent HSC proliferates and progressively

differentiates into hematopoietic progenitors that become increasingly lineage-restricted, ultimately giving rise to mature blood cells.



4-1) Totipotent hematopoietic stem cells (HSCs)

▪ Located in the bone marrow (BM), large in size and rare (about 0.5% of medullary cells); most of them are in a quiescent state (G0 phase of the cell cycle).

▪ Mononuclear cells, morphologically indistinguishable, expressing the CD34 marker.

▪ Properties: totipotency, self-renewal, differentiation, and transplantability.

▪ Under physiological conditions, there is a balance between the production of HSCs by self-renewal and their loss through differentiation.

4-2) Progenitor cells

▪ Represent 0.5 to 1% of bone marrow cells.

▪ Are sensitive to growth factors and are able to form colonies in semi-solid media, referred to as CFUs (Colony-Forming Units).

▪ Are not morphologically identifiable.

▪ Gradually lose their self-renewal capacity and acquire lineage commitment (cellular differentiation).

▪ Two main categories are distinguished:

– early multilineage progenitors (CFU-GEMM, CFU-L),

– late, more committed unipotent progenitors (CFU-G, CFU-M, CFU-MK, CFU-Eo, CFU-B, BFU-E).

▪ Express the markers CD33, CD34 and HLA-DR.

4-3) Precursor cells

▪ Located in the bone marrow and have completely lost any capacity for self-renewal.
▪ Are morphologically identifiable on bone marrow smears (myelogram).
▪ Properties: maturation and proliferation.

Maturation
– Common changes: decrease in cell size, decrease in the nuclear-to-cytoplasmic ratio (N/C ratio), disappearance of nucleoli, and chromatin condensation.
– Lineage-specific changes: nuclear polylobulation, appearance of cytoplasmic granules, and acquisition of specific membrane markers.

Proliferation
– Successive mitotic divisions (depending on the lineage, 3 to 5 mitoses between each precursor stage).

4-4) Mature cells

These include cells of the erythroid, megakaryocytic, granulomonocytic and lymphoid lineages. They are functional, visible and easily identifiable.
These cells leave the bone marrow by a process of endothelial transmigration (bone marrow–blood barrier) and reach peripheral tissues through the bloodstream.
Lymphocytes and monocytes are capable of further differentiation.

Hematopoiesis includes:

▪ **Myelopoiesis**, which leads to the production of myeloid cells:
erythropoiesis (red blood cells), thrombopoiesis (platelets), granulopoiesis (polymorphonuclear cells), and monocytopoiesis (monocytes).

▪ **Lymphopoiesis**, which leads to the production of lymphoid cells.
Lymphoid differentiation is antigen-independent.
The maturation of lymphoid cells into effector cells of the immune response (immunopoiesis) is antigen-dependent.
In adults, **B lymphopoiesis** occurs exclusively in the bone marrow and is antigen-independent.
T lymphopoiesis occurs in the thymus.

5 – Regulation of hematopoiesis

The regulation of hematopoiesis is multifactorial.

5-1) Bone marrow microenvironment ("bone marrow stroma")

It constitutes the supportive and nutritional tissue for hematopoietic cells.
Intercellular communication occurs either through direct cell-to-cell contact or via cytokines.

5-2) Growth factors

Several types of growth factors exist, depending on their site of action during hematopoiesis.

✓ Synergistic (promoting) factors

– Increase the number of cycling hematopoietic stem cells.
– Sensitize hematopoietic stem cells to the action of other growth factors.

Examples: IL-1, IL-4, IL-6, SCF, etc.

✓ Multipotent factors

– Promote the survival and differentiation of early progenitor cells.

Examples: IL-3 and GM-CSF.
IL-3 acts on the lymphoid lineage, whereas GM-CSF acts on the myeloid lineage.

✓ Lineage-restricted factors

– Promote the differentiation of the most committed progenitors, as well as the proliferation and maturation of precursor cells.

Examples: EPO, G-CSF, M-CSF, IL-4, IL-5, etc.

5-3) Negative regulatory factors

They inhibit hematopoiesis in a general or lineage-specific manner:

– Interferons (IFNs), which have antiviral and antimitotic properties,
– Transforming growth factor-β (TGF-β), which inhibits the growth of early progenitors,
– Tumor necrosis factor (TNF),
– Lactoferrin, etc.

5-4) Other factors

▪ Nutrients (iron, vitamin B12, folate / vitamin B9, etc.).
▪ Thyroid hormones, androgens, adrenal hormones and corticosteroids.

6 – Therapeutic use of stem cells present in the bone marrow

In this section, it is preferable to consider not only hematopoietic stem cells, but the bone marrow as a usable tissue.

Hematopoietic stem cells There are three pathological situations in which hematopoietic stem cells (HSCs) are used for therapeutic purposes:

▪ **Allogeneic transplantation** using cells from a healthy related donor, aiming to replace deficient or quantitatively insufficient HSCs.

▪ **Autologous hematopoietic cell transplantation**, not necessarily involving true "stem" cells, used to compensate for the harmful effects of chemotherapy and radiotherapy.
In this context, the approach is mainly "transfusional" and is primarily used in the treatment of solid tumors.

▪ **Replacement of a deficient or missing gene** through gene transfer into autologous hematopoietic stem cells.
At present, this approach is limited to highly selected diseases in which the genetically modified cells have a proliferative advantage.

A.1. Autologous transplantation

An autologous transplant, also called an **autograft**, is a transplant in which the patient receives their own hematopoietic stem cells (HSCs).

Currently, HSCs used for autologous transplants are collected from **peripheral blood**. The use of HSCs derived from bone marrow has become exceptional.

- **Main advantage:** There is **no risk of graft-versus-host disease (GVHD)** because the patient receives their own cells. Therefore, the risk of rejection is null, and the recipient generally recovers faster without the need for immunosuppressive anti-rejection treatments.

A.1.1. Advantages and disadvantages of autologous HSC transplantation

- However, autografts are **not without risks**. The most significant risks are related to the high-intensity chemotherapy and/or radiotherapy administered **before reinfusion of the graft**. These treatments destroy not only cancer cells but also the patient's immune system, which results in a **high risk of infectious complications**.
- The toxicity of conditioning and the risk of **relapse or recurrence** must also be considered. These relapse risks are mainly due to the absence of a **graft-versus-leukemia (GVL) effect**, since the donor and the recipient are the same person. Moreover, the graft collected when the disease is minimal may still contain residual malignant cells.

- **Graft purging techniques** exist to remove tumor cells. These can either **destroy the malignant cells** or **isolate only the HSCs** present in the graft.

A.2. Allogeneic transplantation

In an allogeneic transplant, or **allograft**, the donor and the recipient are two different individuals.

- As in the autologous protocol, the recipient undergoes **chemotherapy and/or radiotherapy** to minimize the disease and ablate their bone marrow.
- The donor HSCs are then injected into the recipient, where they will **reconstitute the immune system** and can fight the disease.

A.2.2. Advantages and disadvantages of allogeneic HSC transplantation

- **Main advantage:** the **graft-versus-leukemia (GVL) effect**. Since the graft comes from a different donor, it contains **T lymphocytes** that can recognize and destroy residual malignant cells in the patient. Thus, the transplanted patient can fight their disease more effectively. Additionally, the grafted cells will rebuild an immune system that the patient retains **long-term**, reducing the risk of rejection over time.
- **Disadvantages:** As with autografts, there is toxicity associated with myeloablative conditioning. Fewer severe side effects are observed with non-myeloablative conditioning. Other risks include rejection, graft-versus-host disease (GVHD), and infections.