Objectives

- state the types of bone marrow
- Identify the major sites of hematopoiesis in the fetus and normal adult.
- outline the compartments of red bone marrow
- describe the types and distinctive characteristic of stem cells
- know the differences between stem cells, progenitor cells, blast cells and mature cells
- Review the stem cell theory of hematopoiesis.
- Discuss growth factors involved in the stimulation and regulation of hematopoietic activity.
- Review maturation stages of each cell lineage, including changes that occur as cells mature.

Bone marrow: Is a soft connective tissue occupies the medullary cavity of long bones and all the spaces between the trabeculae of spongy bone. It accounts for approximately 5% of the body weight in humans

**Two varieties of marrow are recognized**

- Red marrow
- Yellow marrow

Red marrow is the only site for genesis of blood cells in adults and it is the only type found in fetal and young bones but in adult it is restricted to the vertebrae, sternum, ribs, cranial bones and epiphysis of long bones

**Amount of red marrow varies with the age**

- Childhood- red marrow is 100% of bone marrow and present in virtually every bone.
- Adults- red marrow is 50% of bone marrow presenting in sternum, ribs, pelvis and skull.
- 70 years- red marrow reduced to 30% of the bone marrow.

**Yellow marrow**

Consists in main of fat cells which have gradually replaced the other marrow elements.

With an adequate stimulus, yellow marrow may resume the character of red marrow and play an active role in the process of blood development.
Red bone marrow has

✓ A vascular compartment and
✓ An extravascular compartment

**Vascular compartment**
Is supplied by a nutrient artery which branches into central longitudinal arteries which send out radial branches that eventually open into sinuses. These sinuses converge into a central vein that carries the blood out of the bone marrow into the general circulation.
The marrow does not have lymphatic drainage.

**Extravascular compartment**
Is composed of:
1. Stroma of reticular connective tissue
   ✓ reticular tissue (reticular cells, Reticular fibers)
   ✓ macrophages and adipose cells
   ✓ Matrix
2. hematopoietic cords
3. sinusoidal capillaries

**Reticular cells**
Stellate in shape and are in contact with each other along extended cellular processes.
Function of reticular cells:
- Act as a meshwork to support and protect the haemopoietic cells
- Formation of reticular fibers
- Phagocytosis
- They transfer to adipocytes by accumulating lipid in their contents

**Matrix Contain:**
- collagen type I, III
- laminin
- fibronectin and
- proteoglycan

Laminin, fibronectin, and another cell-binding substance, hemonectin, interact with cell receptors to bind cells to the stroma.

The sinusoids are formed by a discontinuous layer of endothelial cells.
Sinusoidal capillaries
An external discontinuous layer of reticular cells and a loose net of reticular fibers reinforce the sinusoidal capillaries.

**Bone marrow barrier:** The blood vessels of the bone marrow constitute a barrier, inhibiting immature blood cells from leaving the marrow. Only mature blood cells contain the membrane proteins required to attach to and pass the blood vessel endothelium. Hematopoietic stem cells may also cross the bone marrow barrier, and may thus be harvested from blood.

**Stem cells**
are pluripotential cells capable of self-renewal. Some of their daughter cells form specific, irreversibly differentiated cell types, and other daughter cells remain stem cells.

They
- retain the ability to renew themselves through mitotic cell division
- can differentiate into a diverse range of specialized cell types.
- found in all multi-cellular organism.

**Two broad types of mammalian stem cells**
1. Embryonic stem cells that are found in blastocysts
2. Adult stem cells that are found in adult tissues

**Embryonic Stem Cells**
are derived from the inner cell mass of the embryo. Because these cells are pluripotent and can virtually form any cell or tissue type, they have the potential for curing a variety of diseases, including diabetes, Alzheimer and Parkinson diseases, anemias, spinal cord injuries, and many others.

**ES cells may be obtained**
1. reproductive cloning.

ES cells may be obtained from embryos after in vitro fertilization. This approach has the disadvantage that
- the cells may cause immune rejection, since they would not be genetically identical to their hosts.
- Another issue with this approach is based on ethical considerations, since the cells are derived from fertilized viable embryos.

2. therapeutic cloning
(somatic nuclear transfer)
take nuclei from adult cells (e.g., skin) and introduce them into enucleated oocytes.
Oocytes are stimulated to differentiate into blastocysts, and ES cells are harvested. Since the cells are derived from the host, they are compatible genetically and since fertilization is not involved, the technique is less controversial.

**Adult Stem Cells**

Adult tissues contain stem cells that also may prove valuable in treating diseases. These cells are restricted in their ability to form different cell types and, therefore, are multipotent, not pluripotent.

Adult stem cells isolated from rat brains have been used to cure Parkinson disease in rats, suggesting that the approach has promise.

**Disadvantages of the approach include**
- the slow rates of cell division characteristic of these cells and
- their scarcity, which makes them difficult to isolate in sufficient numbers for experiments.

**Hematopoiesis**

takes place in the extravascular compartment

The currently accepted theory on how this process works is called the monophyletic theory which simply means that a single type of stem cells gives rise to all mature blood cells in the body.

This stem cells is called the pluripotential stem cells.

It is believed that all blood cells arise from a single type of stem cell in the bone marrow. It is called a pleuripotential stem cell because it can produce all blood cell types.

Pluripotential stem cell proliferate and form

1. lymphoid multipotential cells: one cell lineage that will become lymphocytes.
2. myeloid multipotential cells: another lineage that will form the myeloid cells (granulocytes, monocytes, erythrocytes and megakaryocytes)
Site of hematopoiesis

<table>
<thead>
<tr>
<th>Embryo</th>
<th>yolk sac then liver</th>
</tr>
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<tbody>
<tr>
<td>3rd - 7th months</td>
<td>Spleen</td>
</tr>
<tr>
<td>4th &amp; 5th months</td>
<td>marrow cavity - esp. granulocytes and platelets</td>
</tr>
<tr>
<td>7th month</td>
<td>marrow cavity – erythrocytes</td>
</tr>
<tr>
<td>Birth</td>
<td>mostly bone marrow; spleen and liver when needed</td>
</tr>
<tr>
<td>Birth to maturity</td>
<td>number of active sites in bone marrow decreases but retain ability for hematopoiesis</td>
</tr>
<tr>
<td>Adults</td>
<td>bone marrow of skull, ribs, sternum, vertebral column, pelvis, proximal ends of femurs</td>
</tr>
</tbody>
</table>

Hematopoiesis depends on
- favorable microenvironmental conditions and
- the presence of growth factors.

The microenvironmental conditions are furnished by cells of the stroma of hematopoietic organs, which produce an adequate extracellular matrix.

- A general view of hematopoiesis shows that both the potential for differentiation and the self-renewing capacity of the initial cells gradually decrease.
- the mitotic response to growth factors gradually increases, attaining its maximum in the middle of the process.
- From that point on, mitotic activity decreases, morphological characteristics and functional activity develop, and mature cells are formed

<table>
<thead>
<tr>
<th>stem cells:</th>
<th>Progenitor cells:</th>
</tr>
</thead>
<tbody>
<tr>
<td>This cell can produce all blood cell types</td>
<td>Could be unipotential or bipotential</td>
</tr>
<tr>
<td>Low mitotic activity</td>
<td>High mitotic activity</td>
</tr>
<tr>
<td>Self renewing</td>
<td>Self renewing</td>
</tr>
<tr>
<td>Scarce in the bone marrow</td>
<td>Common in marrow and lymphoid organs</td>
</tr>
<tr>
<td>Cannot be morphologically distinguished (resemble large lymphocyte)</td>
<td>Cannot be morphologically distinguished (resemble large lymphocyte)</td>
</tr>
</tbody>
</table>
Precursor cells (blast): Mature cells:

<table>
<thead>
<tr>
<th>Precursor cells (blast)</th>
<th>Mature cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monopotential cells</td>
<td></td>
</tr>
<tr>
<td>High mitotic activity</td>
<td>No mitotic activity</td>
</tr>
<tr>
<td>Not self renewing</td>
<td></td>
</tr>
<tr>
<td>Common in marrow and lymphoid organs</td>
<td>Abundant in the blood and haematopoietic organs</td>
</tr>
<tr>
<td>Beginning of morphologic differentiation</td>
<td>Clear morphologic differentiation</td>
</tr>
</tbody>
</table>

Hematopoiesis is a compartmentalized process within the hematopoietic tissue:
- Erythropoiesis taking place in distinct anatomical units (erythroblastic islands);
- Granulopoiesis occurs in less distinct foci;
- Megakaryopoiesis occurs adjacent to the sinus endothelium.

Upon maturation, the hematopoietic cells, regulated by the reticular cells, traverse the wall of the venous sinuses to enter the bloodstream:
- Leukocytes, after the action of releasing substances, cross the wall of the sinusoid by their own activity.
- Because erythrocytes (unlike leukocytes) do not have sufficient motility to cross the wall of the sinusoid, they are believed to enter the sinusoid by a pressure gradient that exists across its wall.
- Megakaryocytes form thin processes (proplatelet processes) that cross the wall of the sinusoid and fragment at their tips, liberating the platelets.

MATURATION OF ERYTHROCYTES
Mainly regulated by erythropoietin released by the kidneys; also influenced by androgens:
1. pluripotential cell
2. Myeloid multipotential cell
3. Erythrocyte - colony forming cell
4. Proerythroblast (pronormoblasts): large cell, rounded nucleus coarse chromatin, visible nucleoli, intense basophilia of the cytoplasm
5. Basophilic erythroblast (basophilic normoblasts): condensed nucleus, no visible nucleoli, strongly basophilic cytoplasm because of free ribosomes and polyribosomes.
6. Polychromatophillic erythroblast (polychromatophillic normoblasts) : mixed color cytoplasm purplish blue to grey
7. Orthochromatophillic erythroblast : the amount of haemoglobin is the same as that of erythrocyte. Nucleus with dense and compact chromatin ------- pyknotic extruded from the cell with a thin rim of cytoplasm and plasma membrane

8. Reticulocyte: youngest erythrocyte containing a delicate reticulum the clumped ribosomes responsible for the distinctive staining of the reticulocytes are degraded within 24 hours
9. Erythrocyte : anucleated and biconcave in peripheral blood

Several major changes take place during maturation of erythrocyte
1. cell volume decreases
2. nucleoli diminish in size until they become invisible
3. nuclear diameter decrease and chromatin increase until the nucleus become pyknotic and extruded from the cell
4. gradual decrease in the number of polyribosomes (basophilia) with a simultaneous increase in the amount of haemoglobin (acidophilic protein)
5. mitochondria and other organelles gradually disappear

GRANULOPIOSIS
Regulated by GM-CSF
1. pluripotential stem cells
2. myeloid multipotential stem cells
3. granulocyte colony forming cell
4. Myeloblast:
ovoid nucleus with 2 or more nucleoli, basophilic cytoplasm (abundance of RER and ribosomes)
5. Promyelocyte:
round or oval nucleus, occasionally indented, basophilic cytoplasm containing azurophilic granules (lysosomal enzyme and myeloperoxidase)
6. Myelocyte: appearance of specific granules and the developing myelocytes can be distinguished into 3 types:
   - Neutrophilicmyelocyte
   - Acidophilic myelocyte
   - Basophilic myelocyte
7. Metamyelocyte:
nuclei irregular in shape known as band form, cytoplasm with increasing free
ribosomes, mitochondria and RER.
   Neutrophilic metamyelocyte
   Acidophilic metamyelocyte
   Basophilic metamyelocyte

8. Mature granulocyte:
Neutrophils: horse–shoe or S-shaped nucleus
Acidophil: nucleus with 2 lobes, common in connective tissue of certain
organs (intestine epithelium)
Basophil: nucleus is large, irregular

<table>
<thead>
<tr>
<th>AZUROPHILIC GRANULES</th>
<th>SPECIFIC GRANULES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st appear in promyelocyte</td>
<td>1st appear in myelocyte</td>
</tr>
<tr>
<td>Derived from the inner cisternae of</td>
<td>Derived from an outer cisternae of</td>
</tr>
<tr>
<td>Golgi complex</td>
<td>Golgi complex</td>
</tr>
<tr>
<td>Decrease in number with frequent</td>
<td>Increase in number with maturation</td>
</tr>
<tr>
<td>division and maturation</td>
<td></td>
</tr>
<tr>
<td>They are lysosomes, it’s histochemical</td>
<td>neutrophil contain alkaline</td>
</tr>
<tr>
<td>structure myeloperoxidase and acid</td>
<td>phosphatase and antibacterial lysozyme</td>
</tr>
<tr>
<td>phosphatase</td>
<td>Acidophil contain sulphatase, peroxidase and</td>
</tr>
<tr>
<td></td>
<td>histaminase</td>
</tr>
<tr>
<td></td>
<td>Basophil contain heparin and histamine</td>
</tr>
</tbody>
</table>

MEDICAL APPLICATION
The appearance of large numbers of immature neutrophils (band cells) in the
blood is called a shift to the left and is clinically significant, usually indicating
bacterial infection.

MONOPOIESIS
Maturation of monocytes is regulated by GM-CSF
Maturation of monocytes
1. Pluripotential cell
2. Myeloid multipotential cell
3. Monocyte colony – forming cells
4. Monoblasts
5. Promonocyte:
   - large cell 18 micrometer in diameter, slightly indented nucleus with lacy chromatin and evident nucleoli, basophilic cytoplasm
6. Monocyte:
   - indented nucleus
   - cytoplasm contain large amount of RER, extensive Golgi complex and granule condensation. These granules are primary lysosomes, which are observed as fine azurophilic granules in blood monocytes.
   - Monocytes migrate into the circulation where they remain for about 8 hours before migrating into the connective tissue
   - in the connective tissue they increase in size, acquire multiple lysosomes and become active in phagocytosis (macrophages)
   - life span of macrophages in different tissues may be up to several months

LYMPHOPOIESIS
Maturation of lymphocyte
1. Pluripotential cell
2. Lymphoid multipotential cells: migrate to lymphoid organs
3. Lymphocyte colony forming cell
4. Lymphoblast:
   - large cells, large nucleus with prominent nucleoli, basophilic cytoplasm
   - capable of incorporating \([3H]\) thymidine and dividing two or three times to form prolymphocytes
5. Prolymphocyte:
   - smaller with condensed chromatin, nucleoli less obvious, few azurophilic granules appear in the cytoplasm,
   - no cell surface receptor that mark them as T or B lymphocyte
6. B and T lymphocytes:
   - in the thymus or bone marrow, these cells synthesize cell surface receptors but they are not recognized as distinct cell types using routine histological procedure.
   - They can be recognized by immunohistochemistry
**MEDICAL APPLICATION:**
Leukemias are malignant clones of leukocyte precursors.
They occur in
- lymphoid tissue (lymphocytic leukemias)
- bone marrow (myelogenous and monocyticleukemias).
In these diseases, there is usually a release of large numbers of immature cells into the blood.
The symptoms of leukemias are a consequence of this shift in cell proliferation, with a lack of some cell types and excessive production of others (which are often abnormal in function). The patient is usually anemic and prone to infection.

**THROMBOPOIESIS**
Maturation of platelets is regulated by thrombopoietin (TPO). Mainly produced by the liver
1. **Pluripotential cell**
2. **Myeloid multipotential cell**
3. **Megakaryocyte forming cell**
4. **Megakaryoblast**
   - large cell (15-50 micrometer), large ovoid or kidney shaped nucleus with numerous nucleoli (DNA 30X as much as a normal cell), cytoplasm is homogenous and basophilic
5. **Megakaryocyte**
   - giant cell (35-150 micrometer), irregular lobulated nucleus, coarse chromatin, no visible nucleoli,
   - Cytoplasm contain numerous mitochondria RER, extensive Golgi complex, conspicuous granules contain biologically active substances such as platelet derived growth factor, fibroblast growth factor.
   - The demarkation membranes arise from numerous invaginations of the plasma membrane through out the cytoplasm
   - Electron micrographs indicate that megakaryocytic cytoplasm can fragment through the formation of tiny channels arising from rows of vesicle.
   - The vesicles fuse with their neighbors and establish continuity with the cell membrane, producing an extensive system of tubular platelet demarcation channels that subdivide the cytoplasm into hundreds of platelets each with its covering

**Medical application:** In certain forms of thrombocytopenic purpura, a disease
in which the number of blood platelets is reduced, the platelets appear to be
bound to the cytoplasm of the megakaryocytes, indicating a defect in the
liberation mechanism of these corpuscles. The life span of platelets is
approximately 10 days.

**CLINICAL EVALUATION**

Tests of bone marrow function
- Bone marrow aspiration- cytology and maturation
- Bone marrow biopsy- cellularity and architecture

**Bone marrow transplantation (or hematopoietic stem cell transplantation)**
is a very complicated and risky process, and therefore applied only to patients
with life-threatening diseases (who are resistant to chemotherapy).
Mostly patients with congenital neutropenia, aplastic anemia, sickle-cell
disease, neuroblastoma, lymphoma and many other diseases are treated by
stem cell / bone marrow transplantation.
- **Autologous hematopoietic stem cell transplant**

Autologous HSCT requires the extraction of haematopoietic stem cells (HSC)
from the patient and storage of the harvested cells in a freezer.
The patient is then treated with high-dose chemotherapy with or without
radiotherapy with the intention of eradicating the patient’s malignant cell
population at the cost of partial or complete bone marrow ablation (destruction
of patient's bone marrow function to grow new blood cells).
The patient's own stored stem cells are then returned to his/her body, where
they replace destroyed tissue and resume the patient's normal blood cell
production

- **Allogeneic hematopoietic stem cell transplant**

Allogeneic HSCT involves two people: the (healthy) donor and the (patient)
recipient. Allogeneic HSC donors must have a tissue HLA – human leukocyte
antigen - type that matches the recipient.

**Sources of hematopoietic stem cells**
- **Bone marrow:** in the case of a bone marrow transplant, the HSC are
  removed from a large bone of the donor, typically the pelvis, through a
  large needle that reaches the center of the bone. The technique is
  performed under general anesthesia.
- **Peripheral blood stem cells** are now the most common source of
  stem cells for allogeneic HSCT. They are collected from the blood
through a process known as apheresis. The donor's blood is withdrawn through a sterile needle in one arm and passed through a machine that removes white blood cells. The red blood cells are returned to the donor. The peripheral stem cell yield is boosted with daily subcutaneous injections of Granulocyte-colony stimulating factor, serving to mobilize stem cells from the donor's bone marrow into the peripheral circulation.

- **Amniotic fluid**: It is also possible to extract hematopoietic stem cells from amniotic fluid for both autologous or heterologous use at the time of childbirth.
- **Umbilical cord blood**: Umbilical cord blood is obtained when a mother donates her infant's Umbilical Cord and Placenta after birth. Cord blood has a higher concentration of HSC than is normally found in adult blood.