بسم الله الرحمن الرحيم

Physiology

MID | Lecture 5

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

Leukocytes

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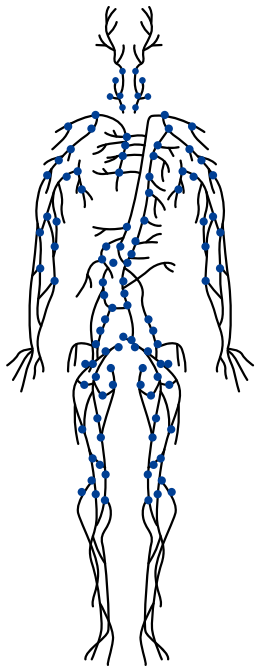
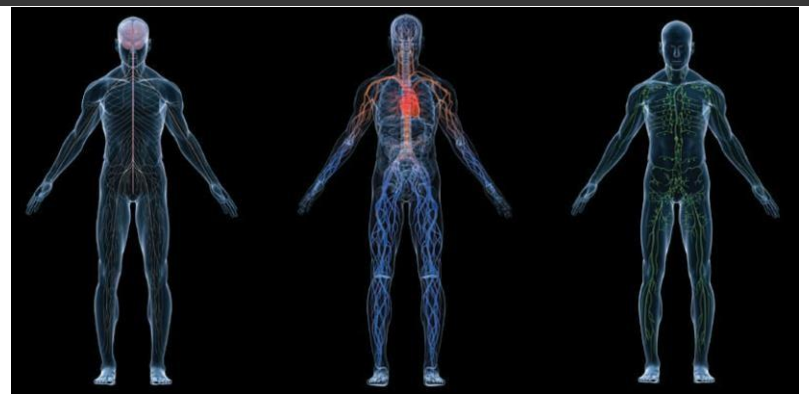


UNIT VI

Chapter 34:



Resistance of the Body to Infection: I. Leukocytes, Granulocytes, the Monocyte- Macrophage System, and Inflammation

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Defense Against Infection "Leukocytes“

- Microorganisms coexist with us and within us (lining surfaces), which can be beneficial or harmful if they invade deep in tissues or the circulatory system.
- There are two main ways for leukocytes to defend our body against microorganisms:
  - Phagocytes can recognize, ingest, and destroy invading organisms and participate in tissue reactions that “wall off” infection.
  - Other white cells get activated (lymphocytes, chapter 35) to mediate responses that destroy or neutralize *specific* microorganisms.

The activated lymphocytes can either:

1. release antibodies which are molecules that can bind to the microorganism making it recognizable to the surrounding tissues or destroy it. (plasma cells)
2. The cells can directly kill the microorganism. (cytotoxic cd8+ cells)

These methods are called acquired immunity

White Blood Cells

- **Circulate in blood and may enter the tissues**

- **<u>Are of six types</u>:**

  - **Polymorphonuclear neutrophils**
  - **“ eosinophils**
  - **“ basophils**
  - **Monocytes**
  - **Lymphocytes (plasma cells)**
  - **Platelets (from megakaryocytes)**
    - They are considered leukocytes because they share the same cellular origin, but their primary function is hemostasis (blood coagulation).

White Blood Cell Counts

- **Total WBC ~ 7,000 / mm^3 in blood**

  (almost 1,000-fold fewer than RBCs)

  - WBCs number can increase upon stimulation from different infections

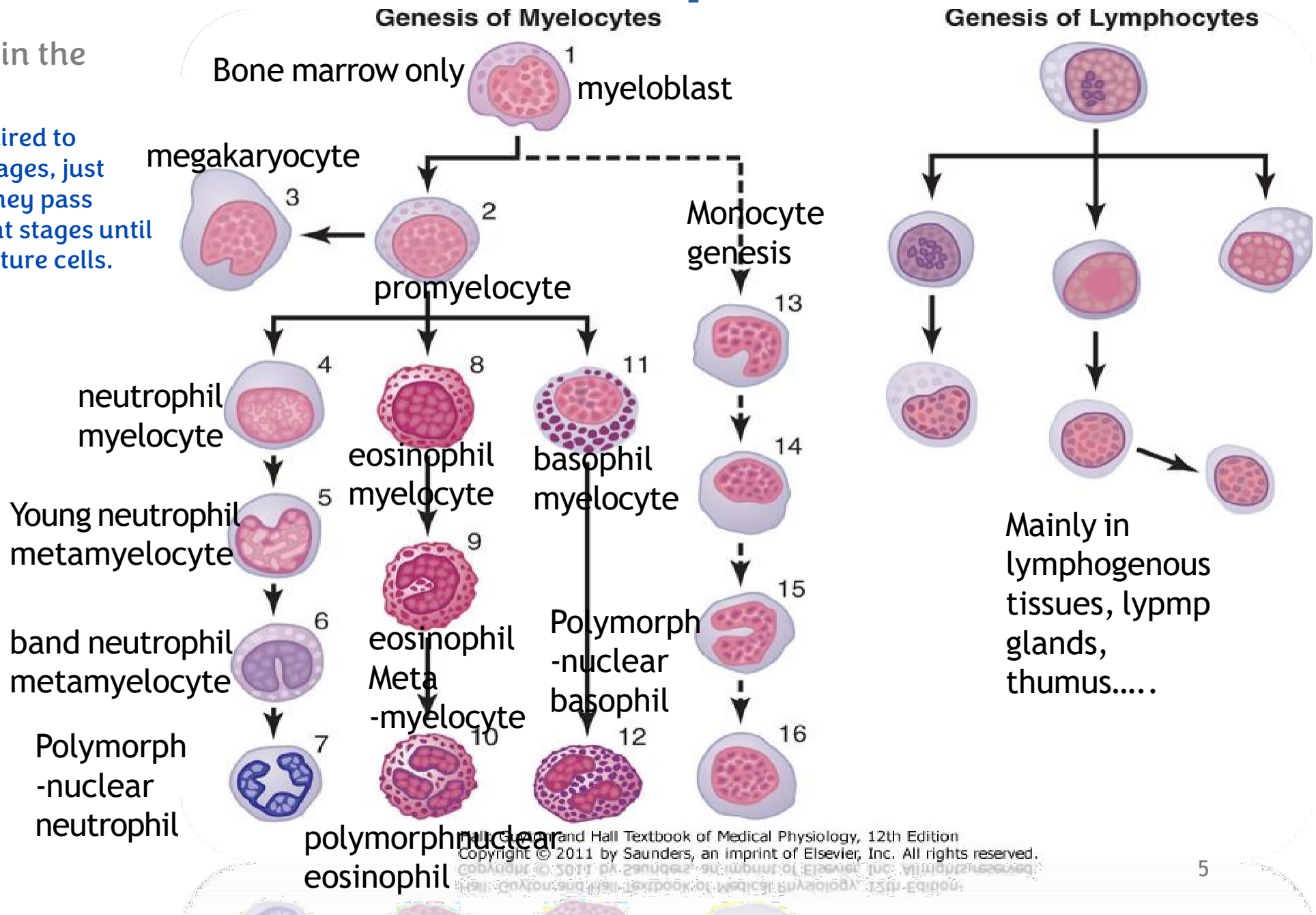
- **Proportions:**
  - **Neutrophils** **62%**
  - **Eosinophils** **2.3%**
  - **Basophils** **0.4%**
  - **Monocytes** **5.3%**
  - **Lymphocytes** **30%**

- **Platelets ~ 300,000 / mm^3**

Leukopoiesis

Explanation in the next slide

You are not required to memorize the stages, just recognize that they pass through different stages until they become mature cells.



Genesis of white blood cells (WBCs)

All blood cells originate from a common stem cell known as the pluripotent hematopoietic stem cell.

White blood cells (WBCs) arise mainly from two lineages:

1. Myeloid lineage:
   This lineage gives rise to *myelocytes*, which develop from *myeloblasts* through several stages of maturation.
   The three types of granulocytes – neutrophils, eosinophils, and basophils – as well as monocytes, all originate from myeloblasts.
   This process occurs primarily in the bone marrow, and once the cells leave the bone marrow, they no longer divide.
2. Lymphoid lineage:
   This lineage develops mainly in lymphoid tissues such as the lymph nodes, thymus, spleen, and partly in the bone marrow.
   Unlike myeloid cells, lymphoid cells can continue to divide even outside the bone marrow.

Genesis of White Blood Cells

- **Granulocytes and monocytes develop in the bone marrow, and most remain there as spare until needed peripherally** (number in marrow ~3x blood; 6-day supply)
- **Lymphocytes develop mostly in the peripheral lymphoid organs** (thymus, spleen, tonsils, lymph nodes, Peyer's patches), less found in blood
- **Megakaryocytes develop and reside in the marrow, fragment to release platelets**

Life Span of White Blood Cells

- **Granulocytes**:
  - Circulating, 4 – 8 hours
  - In the tissues, 4 – 5 days
    (shorter timelines (may be few mins/hrs) with infection, inflammation)

- **Monocytes / Macrophages**:
  - Circulating, 10 – 20 hours
  - Need 8 hours to mature in tissues
  - As tissue macrophages, months or longer
- **Lymphocytes**:
  - Continuously re-circulate from site of origin :

Lymph nodes ➡ blood.. ➡ tissues (diapedesis)

  - Variable: Long-lived… weeks, months, longer (memory cells stay for years)

- **Platelets**: ~ Replaced every ten days~ 30K each day

Neutrophils and Macrophages

Click on the picture to watch the video

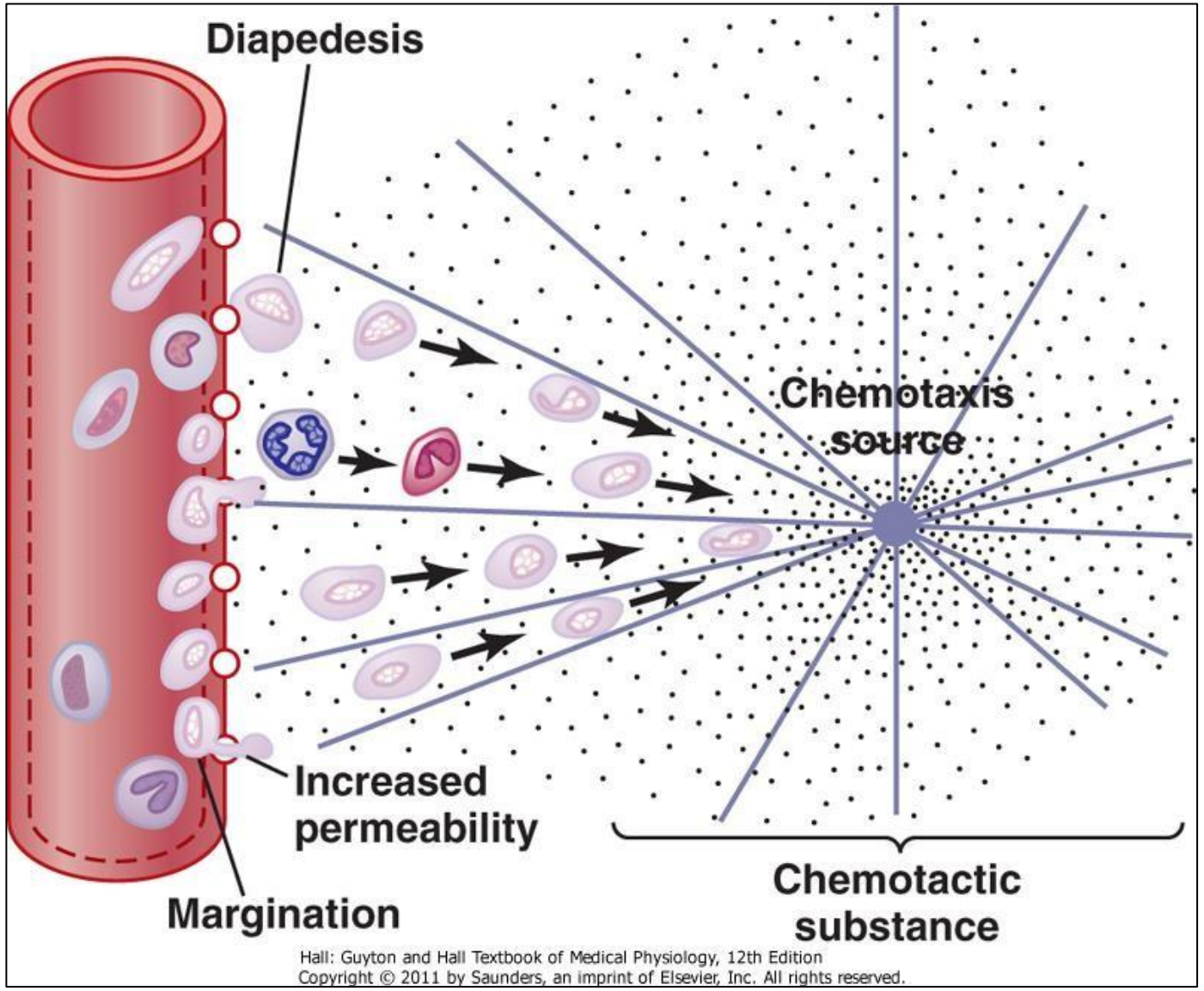
- PMNs and macrophages are considered phagocytes; they perform their function by engulfing and digesting foreign or necrotic particles.

- **Neutrophils are mature cells that can respond immediately to infection**
- **Monocytes mature in the tissues for ~8 hours to become macrophages (monocytes in blood little ability)**
- **Both exhibit motility to the infection site:**
  - **Diapedesis**

  Diapedesis - Medical Animation by Arc Solutions - YouTube
  - **Ameboid motion like amoeba, false legs**
  - **Chemotaxis (Chemoattractants: bacterial or tissue degradation products, complement fragments, other chemical mediators) that act to attract the PMNs or macrophages to the site of infection.**

PMN: polymorphonuclear aka neutrophils

Neutrophil Margination & Migration



The source of infection releases chemoattractants to recruit PMNs and macrophages. Signaling occurs between WBC plasma membrane proteins and endothelial cells, leading to migration (diapedesis) of these cells. Ameboid movement helps them reach the site.

Margination is the process by which WBCs adhere to the endothelial wall. Increased vascular permeability allows them to **extravasate** into the tissue.

Phagocytosis

- **"Phagocytosis" is the ingestion of particles**
- **Phagocytes must distinguish foreign particles from host tissues**
- **Appropriate phagocytic targets (foreign particles):**
  - **May have rough surfaces**
  - **Lack protective protein coats**
  - **May be immunologically marked for phagocytosis by *antibodies* or *complement components* after activation *like C3* that are recognized by receptors on the phagocytes**

    … this immunologic marking is called "opsonization"

Phagocytosis

- **Neutrophils: can ingest 3-20 bacteria**

- **Macrophages: After being activated in the tissues, are extremely effective phagocytes (up to ~100 bacteria)**

- **Macrophages can ingest larger particles…**
  - **Damaged RBCs**
  - **Malarial parasites**

- **Macrophages can extrude digestion products and survive and function for many months unlike neutrophiles that have short life span.**

Digestion of Ingested Particles

- **In both neutrophils and macrophages, *phagosomes* fuse with *lysosomes* and other granules to form *phagolysosomes* (*digestive vesicles*)**

- **These contain *proteolytic enzymes*, and in macrophages, *lipases* (important in killing tuberculosis bacillus and some other bacteria that have lipid coats)**

Bactericidal Agents

- **Bacteria may be killed even if they are not digested**
- **Enzymes in the phagosome or in *peroxisomes* generate strongly bactericidal *reactive oxygen species…***
  - ***Superoxide*** **(O_2^-)**
  - ***Hydrogen peroxide*** **(H_2O_2)**
  - ***Hydroxyl ions*** **(OH^-)**
  - ***The enzyme Myeloperoxidase*** **catalyzes**

$$H_2O_2 + 2\ Cl^- \longrightarrow 2\ H^+ + 2\ ClO^-$$

  - Hydrogen peroxide combines with chloride (via myeloperoxidase) to form hypochlorous acid (HOCl), a highly reactive antimicrobial compound.

- These compound are made in the peroxisomes

The Reticuloendothelial System

"The monocyte-macrophage system"

- **After entering the tissues, macrophages become fixed and may be resident for years**
- **When appropriately stimulated they can break away and move to sites of inflammation**
- **Circulating monocytes, mobile macrophages, fixed tissue macrophages, and some specialized endothelial cells form the *reticuloendothelial system*, almost all derived from monocytes, comprising a phagocytic system located in all tissues**

Specialized macrophages

Macrophages are widely distributed to prevent microorganisms from entering the blood.

- **Skin:** Microorganisms are intercepted by histiocytes (Langerhans cells), resident macrophages that prevent their spread.

- **Lymph nodes:** Any organism in the tissues is captured by lymph nodes, where macrophages filter and destroy it.

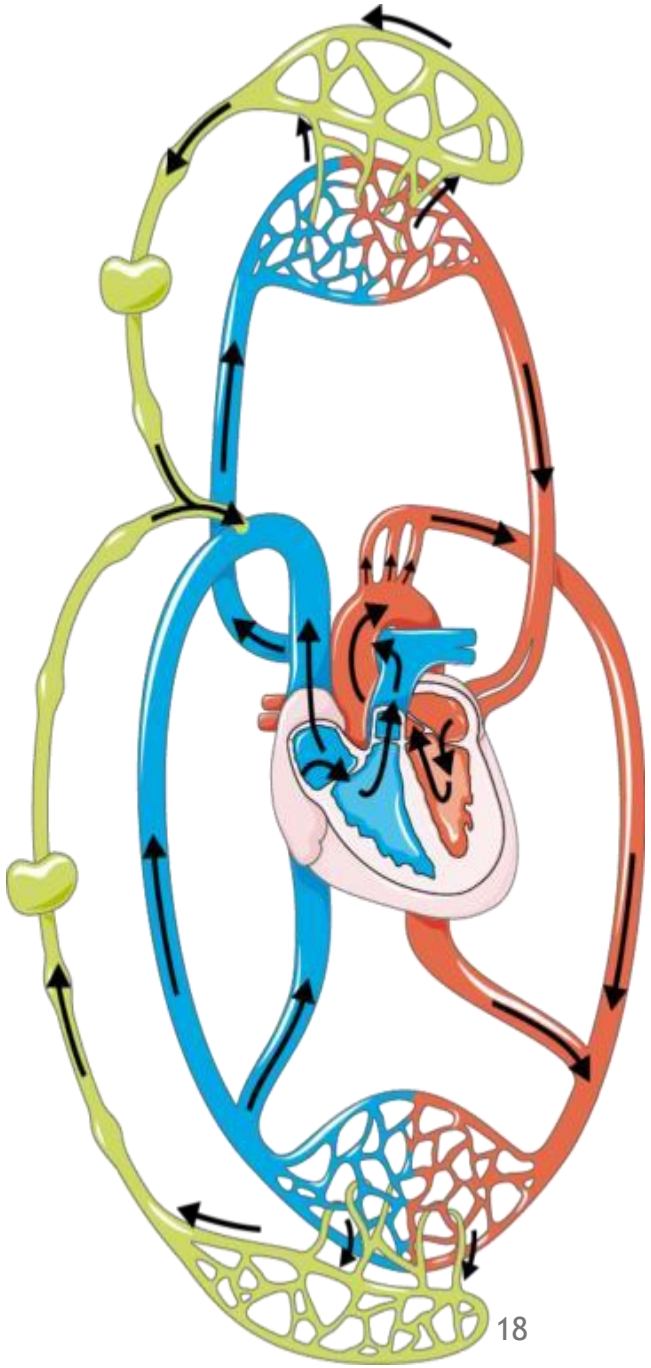
- **Alveoli:** Pathogens and particles like silica are blocked by alveolar macrophages, preventing entry into the bloodstream.

- **Liver:** Microorganisms from the GI tract reach the liver via the portal circulation and encounter Kupffer cells before entering the blood.

- **Blood:** If pathogens enter the circulation, the spleen and resident macrophages in the bone marrow act to eliminate them.

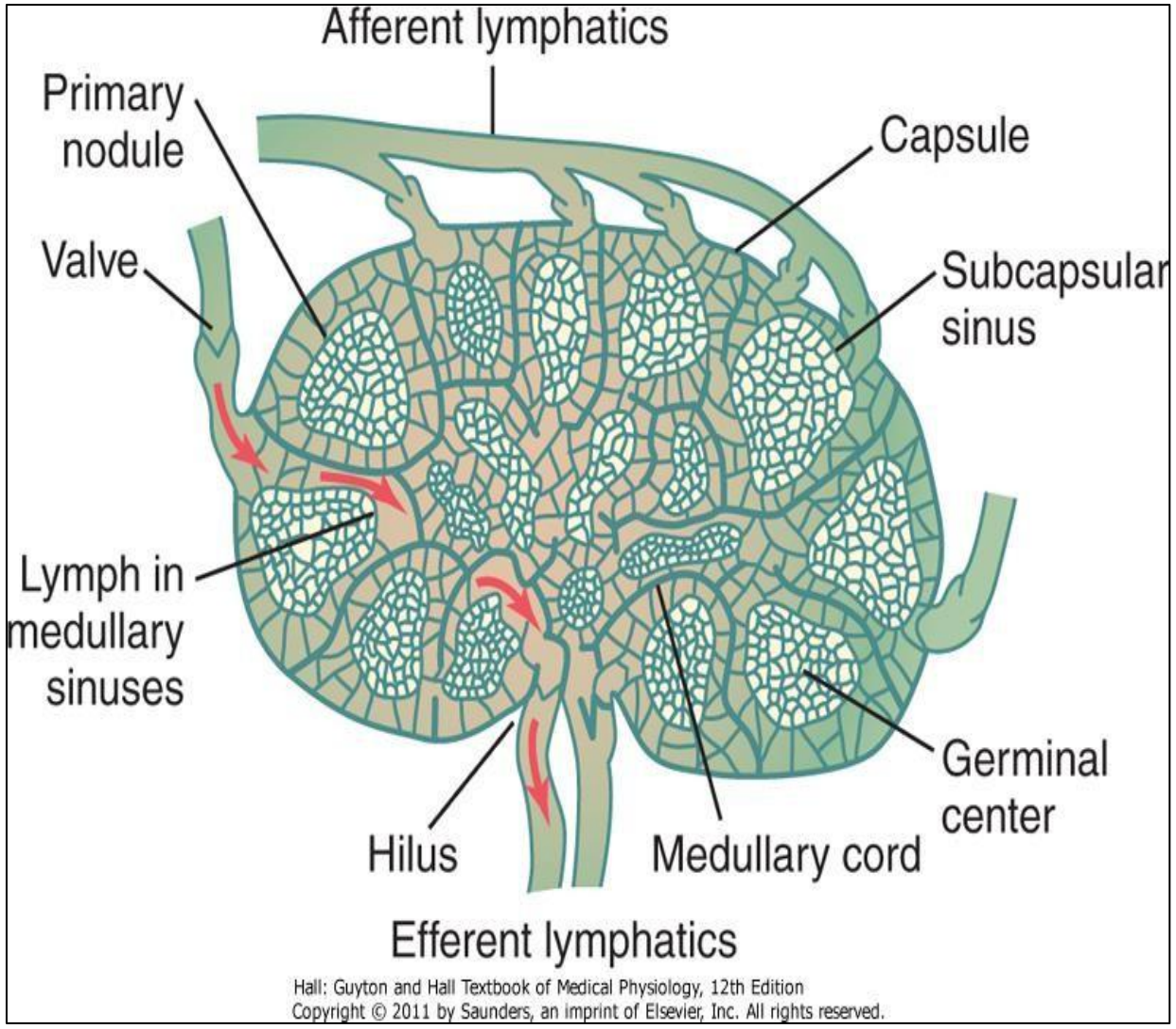
Specialized Macrophages

- Skin, subcutaneous (histiocytes)
- Lymph nodes
  - Ingest / sample particles arriving through the lymph
- Alveolar macrophages
  - Digest or entrap inhaled particles and microorganisms like silica, tuberculosis bacilli.
- Kupffer cells
  - Lining sinusoids, Surveillance of the portal circulation.
- Macrophages in the spleen and bone marrow
  - Surveillance of the general circulation

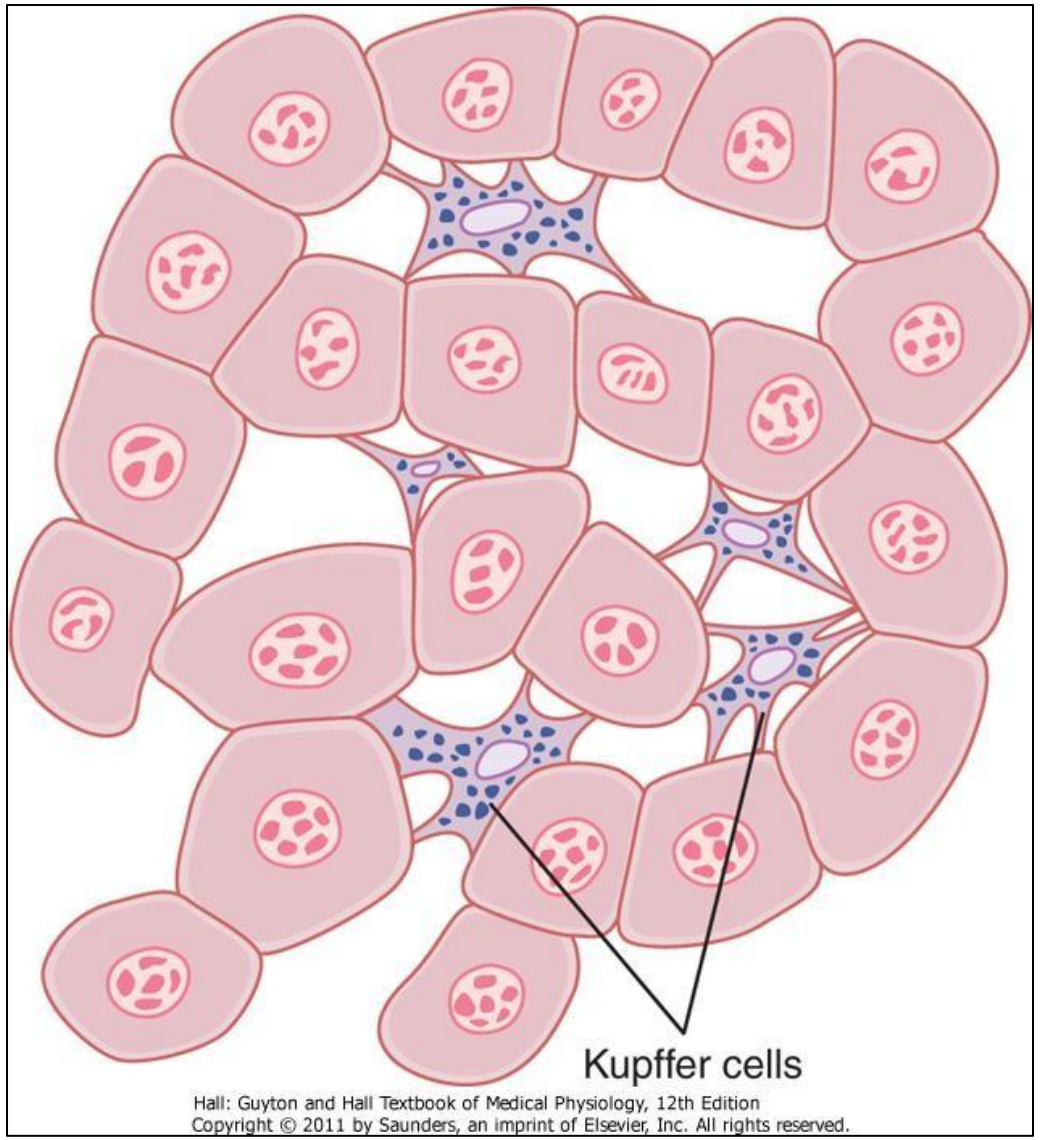


Structure of a Lymph Node

It is a meshwork that acts as a filter, with macrophages preventing microorganisms from entering the blood circulation.

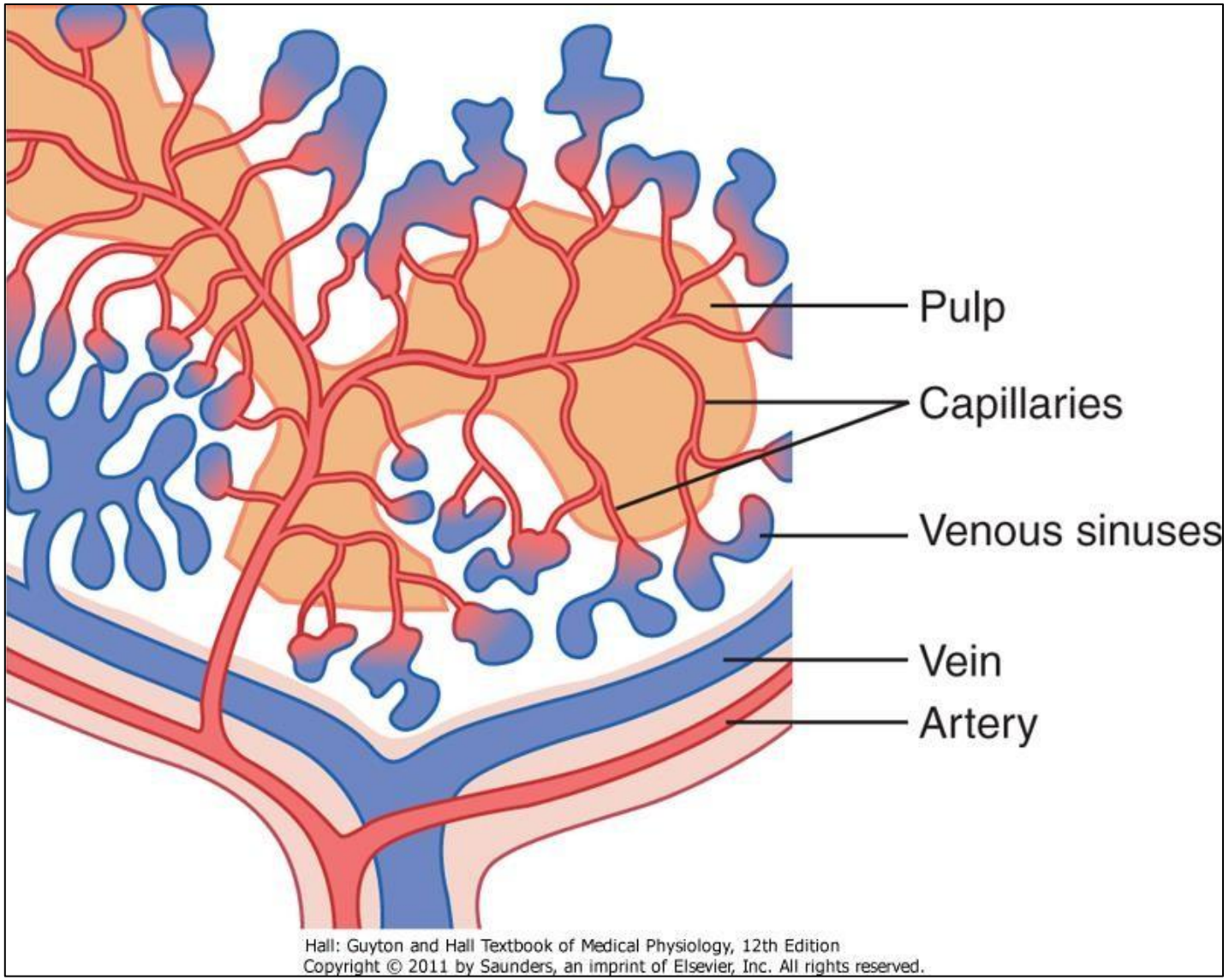


Kupffer Cells in the Liver Sinusoids



Structure of the Spleen

Resembles the structure of lymph nodes: the red pulp and venous sinuses contain macrophages that line them to capture microorganisms.



Neutrophils, Macrophages & Inflammation

- **Inflammation is a cascade of events driven by chemical mediators and characterized by heat, redness, swelling, and pain**
- **Physiologically, it involves…**
  - **Vasodilatation and increased blood flow**
  - **Increased capillary permeability increasing the swelling**
  - **Coagulation of interstitial fluids**
    - **Coagulation of interstitial fluid occurs when fibrinogen leaks from blood into the interstitial space, forming a gel that walls off the infection and helps prevent its spread.**
  - **Accumulation of granulocytes and monocytes**
  - **Swelling of tissue cells**
- **Mediators:** ***histamine, bradykinin, serotonin, prostaglandins, complement products, clotting components, lymphokines***

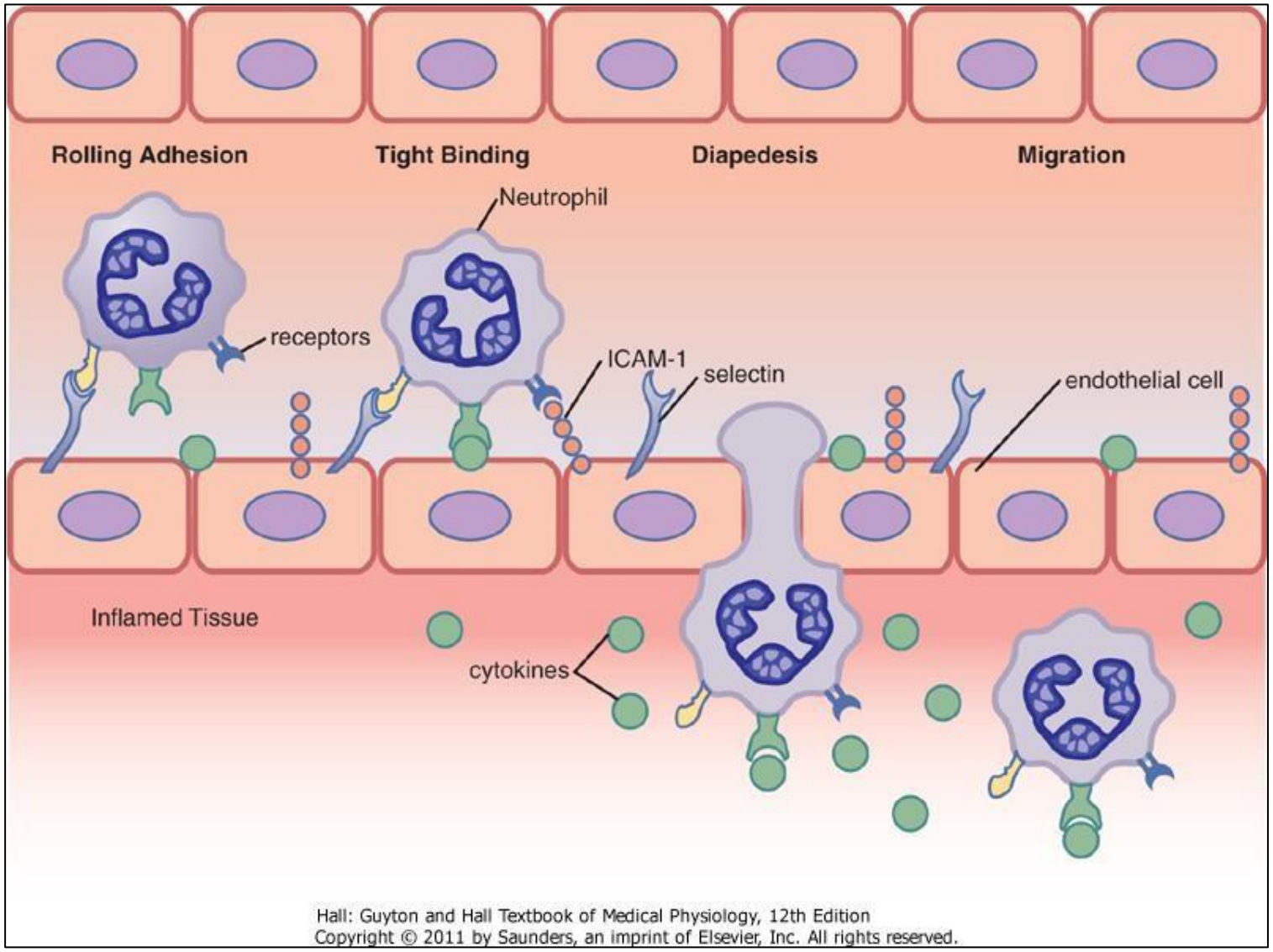
“Walling Off” Sites of Inflammation

- **Fibrinogen clots and other mediators minimize fluid flow in and out of the inflamed area**
- ***Staphylococci* cause intense inflammation being more toxic are effectively “walled off”**
- ***Streptococci* induce less intense inflammation and may be more likely to spread than *staphylococci, and cause death***

Neutrophils and Macrophages in Inflammation

- Tissue macrophages that encounter foreign particles enlarge and become mobile to provide a first line of defense (min)
- Within an hour neutrophils migrate to the area in response to inflammatory cytokines (TNF, IL-1) released from the macrophages.
  2nd line of defense
- Upregulated *selectins* and *ICAM-1* on endothelial cells
- Bind to *integrins* on neutrophils, leading to *margination*, followed by ***diapedesis***, and ***chemotaxis*** directing neutrophils into the inflamed tissues, to kill bacteria and scavenge
  - blood vessels are always located within ≤50 μm from tissues, and chemotaxis is effective up to ~100 μm.

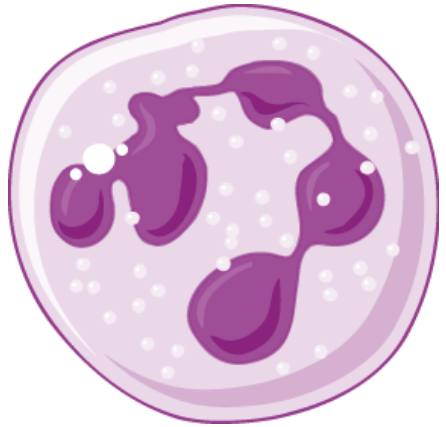
Neutrophil Migration to an Inflamed Site



- In response to increased cytokines, ICAM-1 and selectins (adhesive molecules) are upregulated at the infection site. They bind to complementary proteins on neutrophils, allowing neutrophils to squeeze between endothelial cells and enter the tissues.

- Integrins on neutrophiles
- ICAMS and selectins on endothelium

Neutrophilia



- **With intense inflammation neutrophil count can increase dramatically…**

4,000-5,000 ⟶ 15,000-25,000

3-4x
shortly after the beginning of inflammation

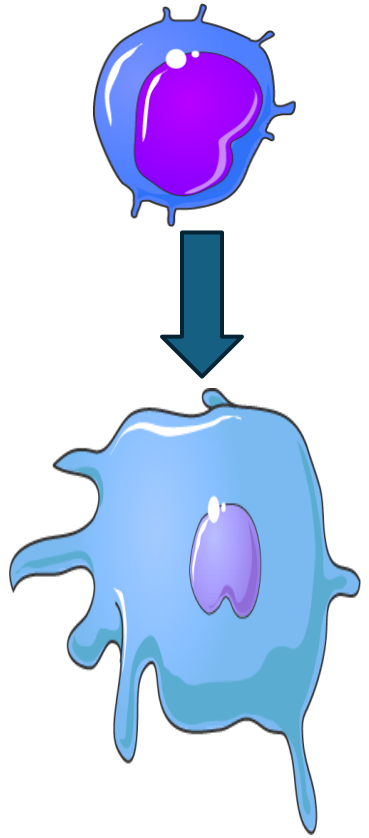
- **Results from mobilization of mature neutrophils from the bone marrow by inflammatory mediators (TNF & IL)**

Secondary Macrophage Invasion

- The third line (migration of monocytes) called so because of the slow progression and maturation of these cells.

- **In response to chemoattractants, monocytes gradually accumulate (slowly) and become macrophages (after ~ 8 hours mature)**

- **In part due to increased bone marrow production (store is low), macrophages become the dominant inflammatory cell over <u>several weeks</u>, cleaning up remaining bacteria, necrotic tissue, and directing tissue remodeling. Third line of defense**



After a few weeks, monocytes (macrophages) dominate the inflammation because they are the most effective cells for clearing and cleaning the inflamed sites.

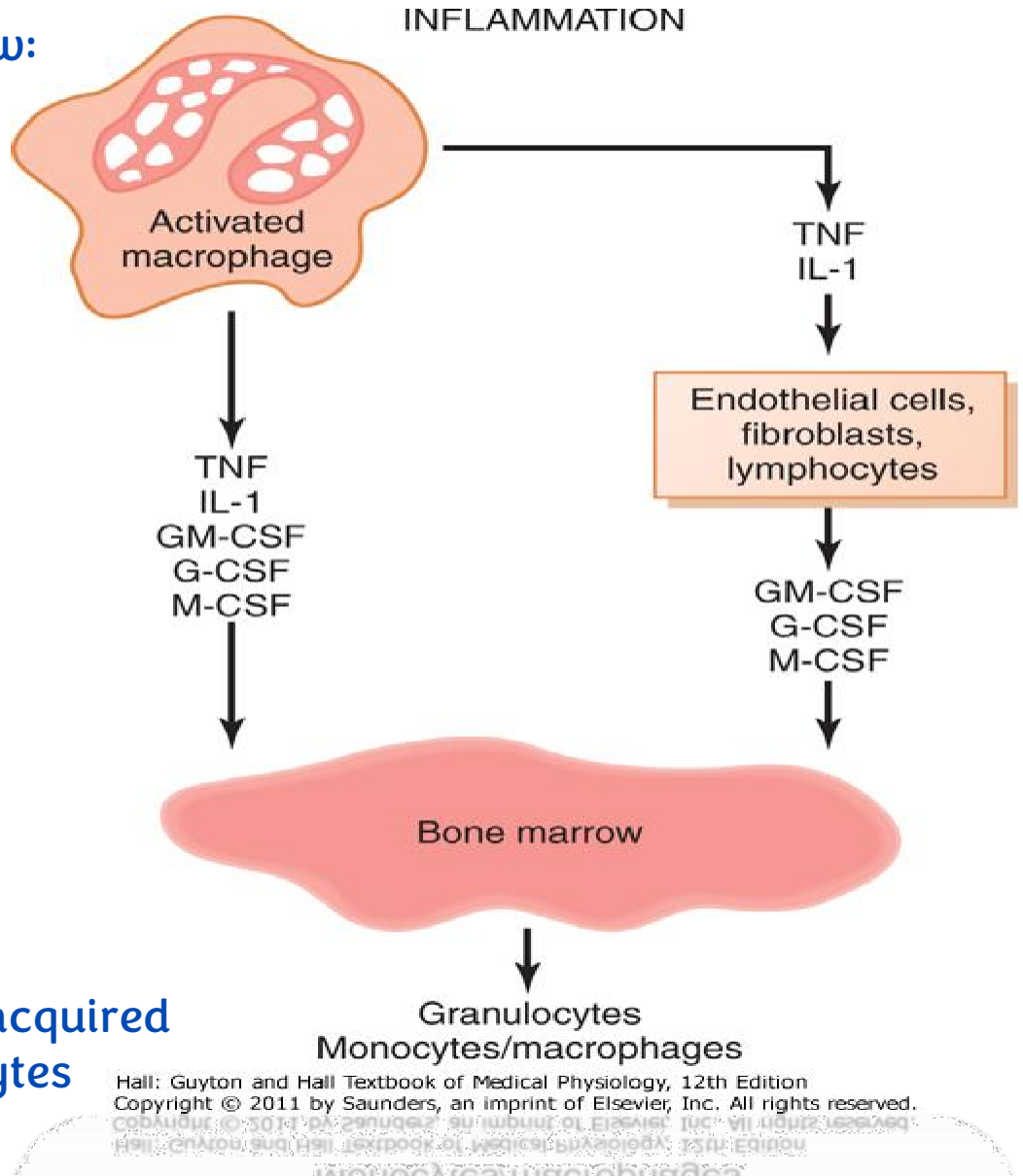
Bone Marrow Responses

- Bone marrow is stimulated by chemical mediators such as IL-1, CSF, and TNF.
- **Growth factors produced in response to infection and inflammation drive proliferation and differentiation of leukocyte precursors in the marrow**
- **First mature cells released after 3 – 4 days after stimulation of the BM.**
- **The bone marrow can increase production of granulocytes and monocytes by 20 – 50- fold and maintain this for months or years**
- **Fourth line of defense**

Bone Marrow Response to Inflammation

- Two ways to stimulate bone marrow:

1. This illustrates the feedback between activated macrophages at the infection site and the bone marrow. Cytokines such as TNF, IL-1, GM-CSF, G-CSF, and M-CSF are released into the blood to stimulate the bone marrow to produce more granulocytes and monocytes.



2. TNF and IL-1 may also activate other cells—such as endothelial cells, fibroblasts, and lymphocytes—to produce these cytokines, but the contribution from activated macrophages is more significant.

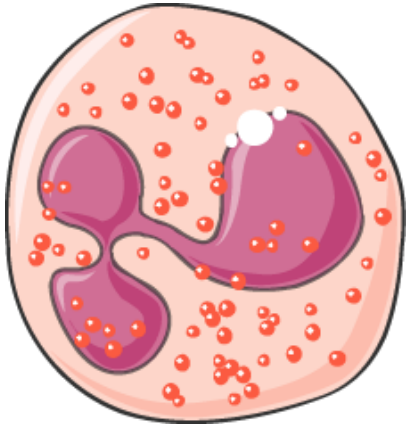
Activated macs are also important in acquired immunity to activated other lymphocytes

Formation of Pus

- **Pus is composed of dead bacteria and neutrophils, many dead macrophages, necrotic tissue that has been degraded by proteases, and tissue fluid, often in a cavity formed at the inflammatory site**

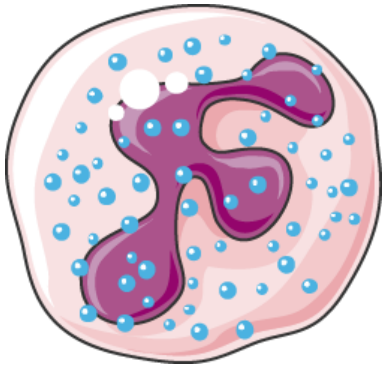
- **Over days and weeks it is absorbed into the surrounding tissue and lymph and disappears**

Eosinophils



- Eosinophils are weak phagocytes, they have granules and exhibit chemotaxis
- Particularly important in defense against parasites, Ex: schistosomiases and trichinosis
- Can adhere to parasites and release substances that kill them (hydrolases, reactive oxygen species, major basic protein(larvacidal).
- Also accumulate in tissues affected by allergies, perhaps in response to eosinophil chemotactic factor from basophils (eosinophils may detoxify some products of basophils)

Basophils

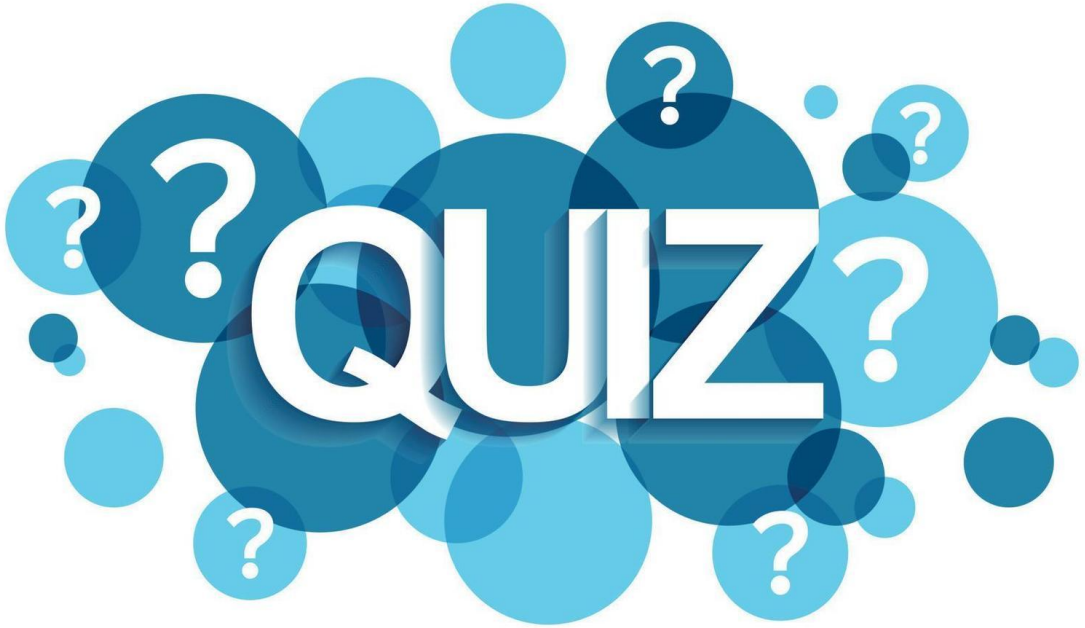


- **Similar to *mast cells* adjacent to Capillaries, both cell types release heparin**

- **Basophils and mast cells both release histamine, bradykinin, and serotonin which are involved in aggravating the inflammatory reaction.**

- **When IgE bound to receptors on their surfaces is cross-linked by its specific antigen, mast cells and basophils *degranulate*, releasing…**
  - ***histamine, bradykinin, serotonin, heparin, leukotrienes,* and several *lysosomal enzymes***

Physiology Quiz 5



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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			