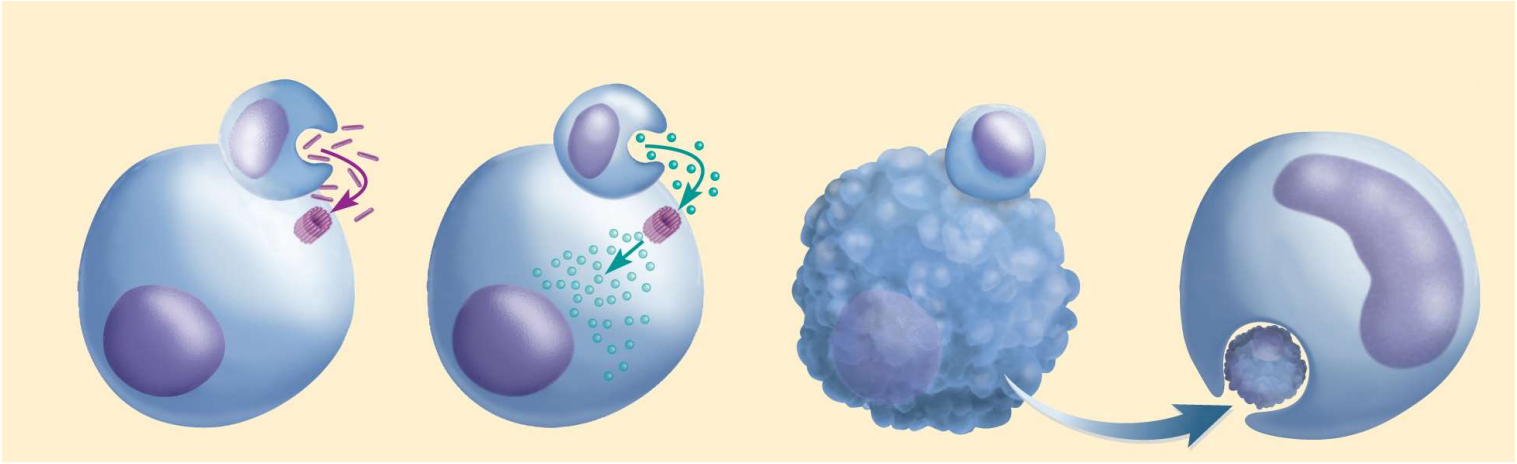
An Introduction to the "Three Lines of Defenses" Against Pathogens

---

Review of the "First & Second Lines of Defenses" Protecting Us Against Pathogens



Three Defenses Against Pathogens

- What are pathogens? (*microorganism capable of producing disease in a healthy person)*
- Hos is tissue damaged? (*infectious organisms (bacteria, virus, parasites), toxins, chemicals, physical trauma, and radiation)*
- *Human host must defend themself against <u>internal and external threats</u> that damage their tissue – use three separate "lines of defenses"*
  - First Line – physical barriers
  - Second Line – non specific resistance
  - Third Line – adaptive immunity

These are innate

Three Defenses Against Pathogens

- First line of defense = external barriers = Skin + mucous membranes // called innate defense – present at birth

- Second line of defense – provides non-specific resistance to pathogens

    - Also called innate because present at birth

    - *leukocytes and macrophages, antimicrobial proteins, immune surveillance cells, inflammation, and fever*

    - effective against a broad range of pathogens / but not specific pathogens!

- Third line of defense (acquired immunity – requires activation)

    - defeats “specific” pathogens

    - leaves body with ‘memory’ – secondary response to pathogen

    - cellular and humoral response (location of pathogen require different responses)

What is acquired immunity?

- **Acquired immunity = Third Line of Defense**
  - Specificity - able to recognize specific species of pathogen
  - Memory – stores record of first exposure // ensures rapid response after first exposure (minutes not days!)
  - There is a “Humoral Response” /// if pathogen outside our cells /// rendered harmless and tagged for destruction by antibodies

What is acquired immunity?

- **Acquired immunity = Third Line of Defense**
  - There is a “Cellular Response” /// if pathogen inside our cells // adaptive immunity kills infected cell
  - Able to learns from prior exposure so faster response occurs in second exposure to same pathogen (ie. The memory)
  - The three “R”s of immunity: recognize / react / remember

First Line of Defense

Physical Factors	Chemical Factors
Epidermis of skin	Sebum
Mucous membranes	Lysozyme
Mucus	Gastric juice
Hairs	Vaginal secretions
Cilia	
Lacrimal apparatus	
Saliva	
Urine	
Defecation and vomiting	

Second Line of Defense

Antimicrobial Substances	Cellular & Physiologic
Interferons	Natural killer cells
Complement system	Phagocytes
Iron-binding proteins	Inflammation
Antimicrobial proteins	Fever
	TOLL Like Receptors / PAMP

The External Barriers

- **Skin**
  - makes it mechanically difficult for microorganisms to enter the body
  - toughness of keratin
  - too dry and nutrient-poor to support microbial growth
  - **defensins** – peptides that kill microbes by creating holes in their membranes
  - **acid mantle** – thin film of lactic acid from sweat which inhibits bacterial growth

The External Barriers

- Mucous membranes
  - digestive, respiratory, urinary, and reproductive tracts are open to the exterior and protected by mucous membranes
  - mucus physically traps microbes
  - lysozyme - enzyme destroys bacterial cell walls
- Sub-epithelial areolar tissue
  - viscous barrier of hyaluronic acid
  - hyaluronidase - enzyme used by pathogens to make hyaluronic acid less viscous

The External Barriers

- Mucous membranes
  - digestive, respiratory, urinary, and reproductive tracts are open to the exterior and protected by mucous membranes
  - mucus physically traps microbes
  - lysozyme - enzyme destroys bacterial cell walls
- Sub-epithelial areolar tissue
  - viscous barrier of hyaluronic acid
  - hyaluronidase - enzyme used by pathogens to make hyaluronic acid less viscous

Second Line of Defense / Cellular Contributions

(Review Function of Formed Elements)

Granulocytes
- Neutrophils*
- Eosinophils*
- Basophils (mast cells)

Agranulocytes
- Monocytes (macrophage)*
- lymphocytes (T cells / B cells / NK cells)

- Phagocytes provide the cellular component
  - Able to engulf bacteria, endocytocytosis
  - Bacteria, infected cells, and fragments of cells
  - internalized as phagosome / fuse with lysosomes
  - Neutrophils kill bacteria and eosinophils kill parasites with respiratory burst

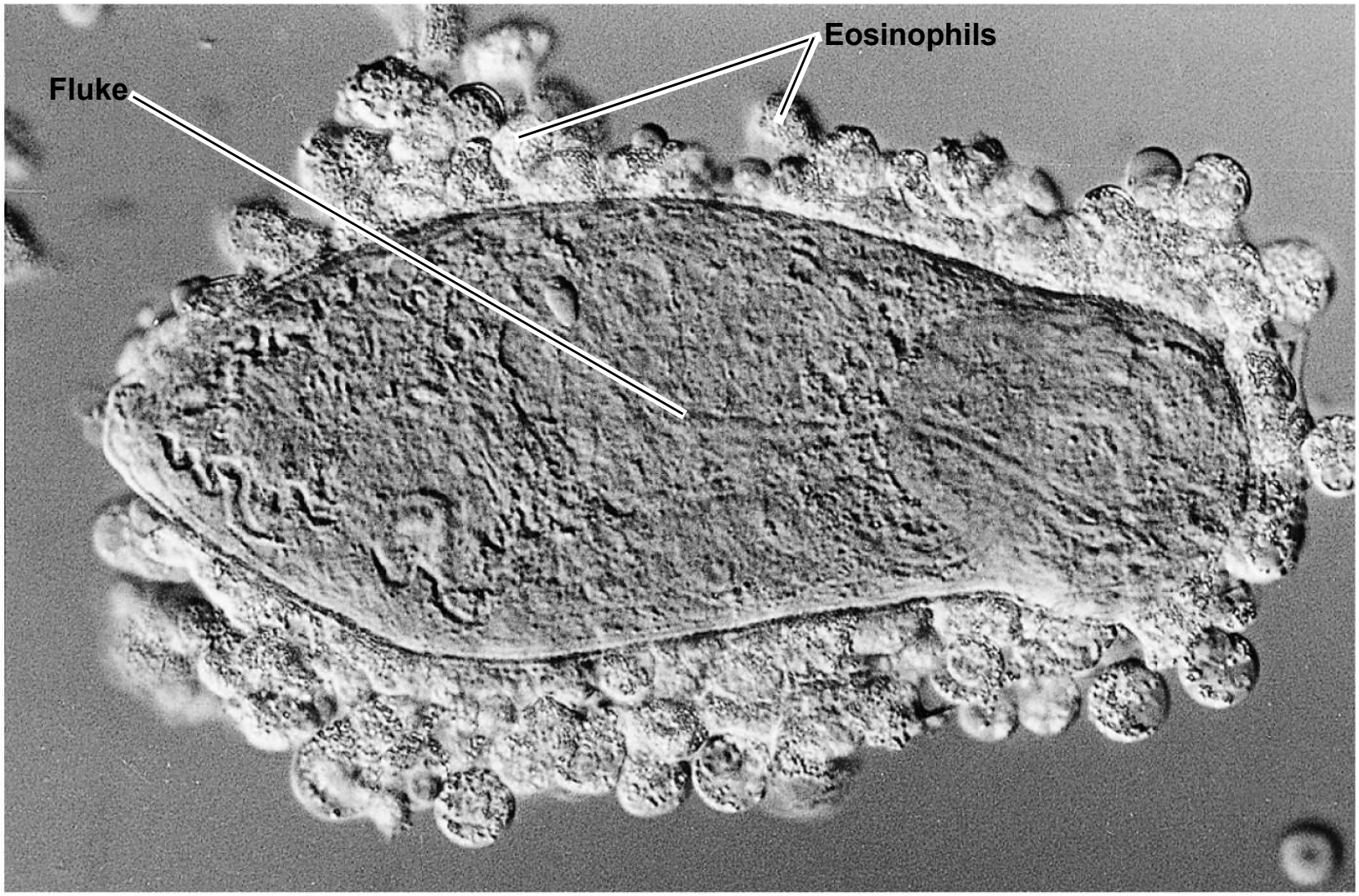
Neutrophils

- Wander about within the connective tissues of your body to seek and kill bacteria
  - Responsible for phagocytosis and digestion
  - Create a killing zone
    - produces a cloud of anti-bacterial chemicals
    - Degranulation // lysosomes discharge into tissue fluid
    - respiratory burst – neutrophils rapidly absorb oxygen
    - toxic chemicals are created ($O_2^{\cdot -}$, H_2O_2, HClO) /// Free radical of oxygen, hydrogen peroxide, hypochlorite
    - kill more bacteria with these toxic chemicals than by phagocytosis

Eosinophils

- found especially in the mucous membranes
- stand guard against parasites, allergens (allergy causing agents), and other pathogens
- kill tapeworms and roundworms by producing superoxide, hydrogen peroxide, and toxic proteins
- promote action of basophils and mast cells
- phagocytize antigen-antibody complexes
- limit action of histamine and other inflammatory chemicals
- also create a "respiratory burst" similar to neutrophils

Antibody-dependent cell-mediated cytotoxicity (ADCC).

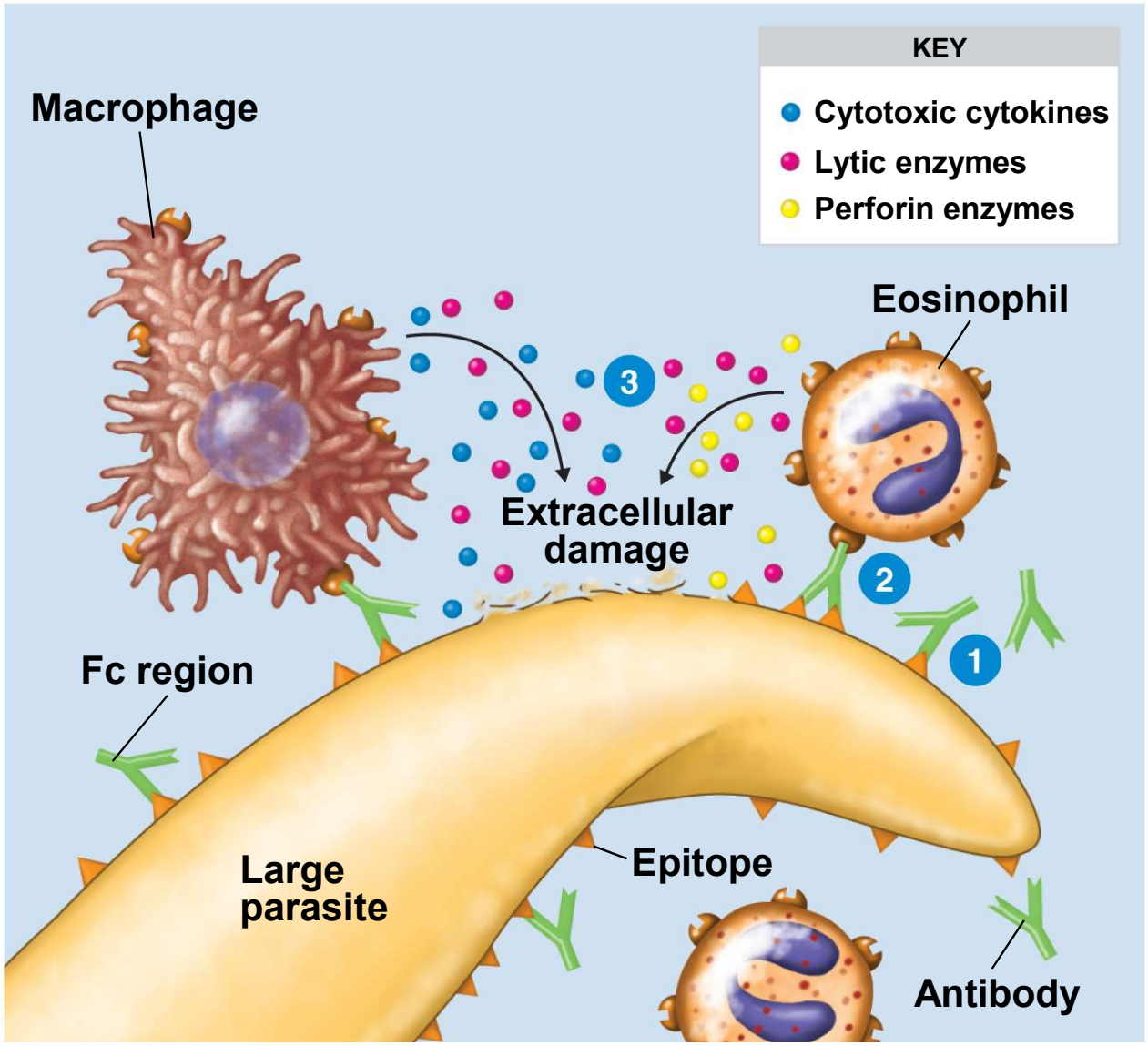


(b) **Eosinophils adhering to the larval stage of a parasitic fluke.**

SEM

20 μm

Antibody-dependent cell-mediated cytotoxicity (ADCC).



Organisms, like some parasites to large to be ingestion by phagocytic cells, must be attacked and eliminated by extracellular molecules.

Basophils Change into Mast Cells

- secrete chemicals that aid mobility and action of other leukocytes // initiates inflammation
  - leukotrienes – activate and attract neutrophils and eosinophils
  - histamine – a vasodilator which increases blood flow // peeds delivery of leukocytes to the area
  - heparin – inhibits the formation of clots // would impede leukocyte mobility
  - basophils become mast cells //// after basophil leave blood and lodge themselves into the CT throughout body
  - secrete similar substances
  - IgE become mast cell membrane receptors / antigen binding results in degranulation of mast cell.

About Lymphocytes – 3 Types

- Three basic categories
- Circulating blood contains
  - 80% **T cells (cellular immunity)**
  - 15% **B cells (humoral immunity)**
  - 5% **NK cells (surveillance)**
- Many diverse functions
- We will look at the function of T and B lymphocytes as part of Acquired Immunity - However
- NK Cells // part of 2nd line - because they perform general surveillance for cells infected with cancer or virus // NK Cells do not "specifically" recognize and attack infected cells – this is why we refer to NK cells as "immune surveillance"

Monocytes to Macrophage

- emigrate from the blood into the connective tissue
- change from monocyte to macrophage
- monocytes secrete many different types of cytokines which regulate inflammation and immunity while they are in blood
- macrophage system – include all the body's avidly phagocytic cells, include not only the "macrophage" (Note: neutrophils , B cells, and esinophils also phagocytic)
- wandering macrophages – actively seeking pathogens // widely distributed in loose connective tissue
- resident "fixed macrophages" = phagocytize only pathogens that come to them
    - microglia – in central nervous system
    - alveolar macrophages – in lungs
    - hepatic macrophages – in liver

NK Cells - Immune Surveillance

- Immune surveillance – a phenomenon in which natural (NK) killer cells continually patrol the body on the lookout for pathogens and diseased host cells.

- Natural killer (NK) cells attack and destroy:

  - Primary role to kill cells infected with virus and cancer cells

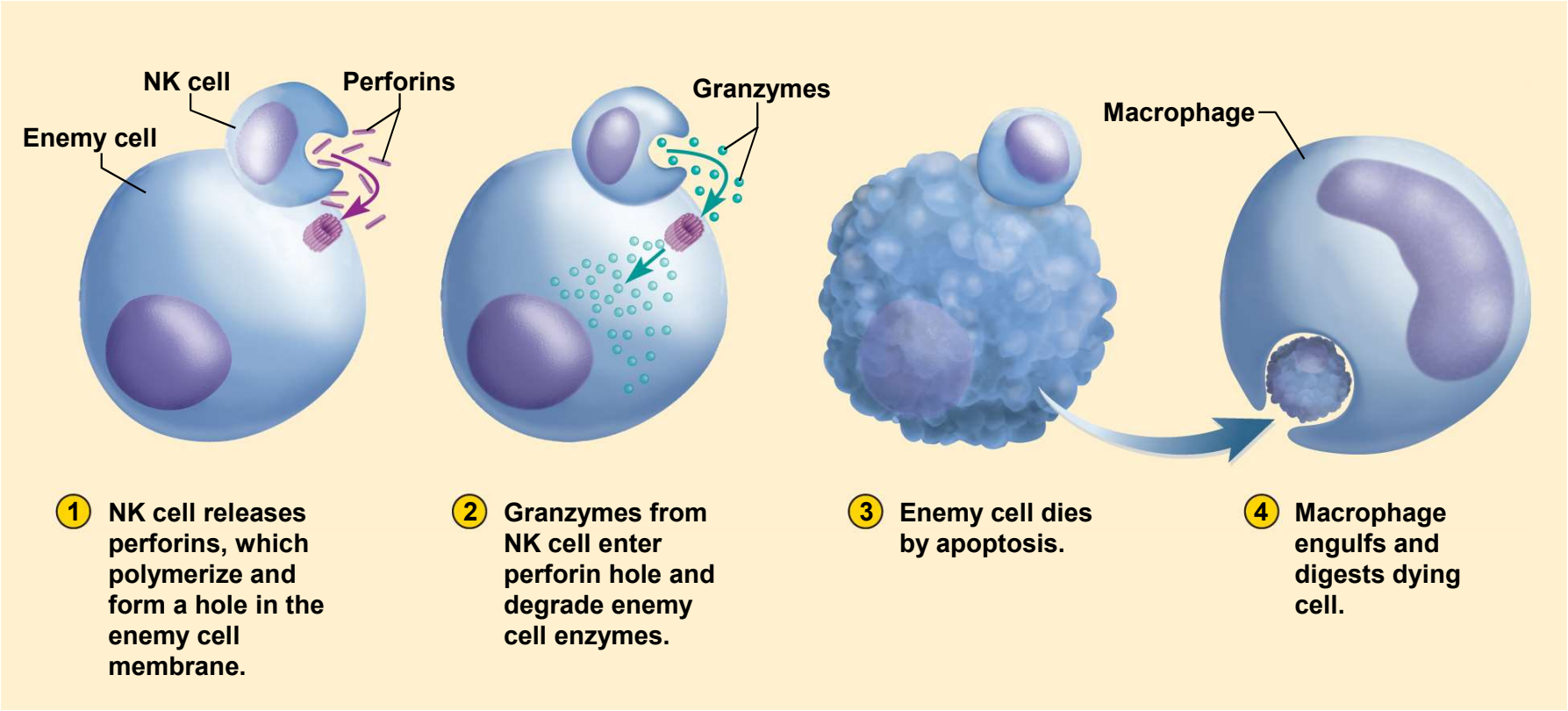
    - recognizes enemy cell
    - NK cells bind to it
    - release proteins called perforins

      - polymerize a ring and create a hole in its plasma membrane

      - secrete a group of protein degrading enzymes – granzymes

      - enter through pore and degrade cellular enzymes and induce apoptosis

Action of NK cell



Note: same mechanism used by cytotoxic T cells to kill infected cells in specific immunity!

Toll Like Receptors and Pathogen Associated Molecular Patterns (This is part of the 2nd line)

- TLR // Receptors on macrophage, neutrophils, and epithelial cells lining mucous membranes (e.g. respirtaory and GI tracts)
- 11 different types of human TLR
  - Each one recognizes a different "class" of attacking microbe (e.g. gram negative bacterial like salmonella but same receptor would also bind to all gram negative bacteria)
  - Once bound to a PAMP the TLR activates the release of inflammatory chemicals called "cytokines" from monocyte or similar cell.
- Pattern Associated Molecular Patterns (PAMP = pathogen associated molecular patterns) – on bacteria and viruses
  - Macrophage, dendritic cells, endothelial cells, lymphocytes have TLR matched to PAMP on bacteria and viruses

Non-Cellular Antimicrobial Proteins
Part of the Second Line of Defense

- Two families of antimicrobial proteins inside internal tissues of body
  - **interferons //** *proteins that inhibit viral reproduction – secreted by cells infected by virus*
  - **complement system //** *provide short-term, nonspecific resistance to pathogenic bacteria and viruses*

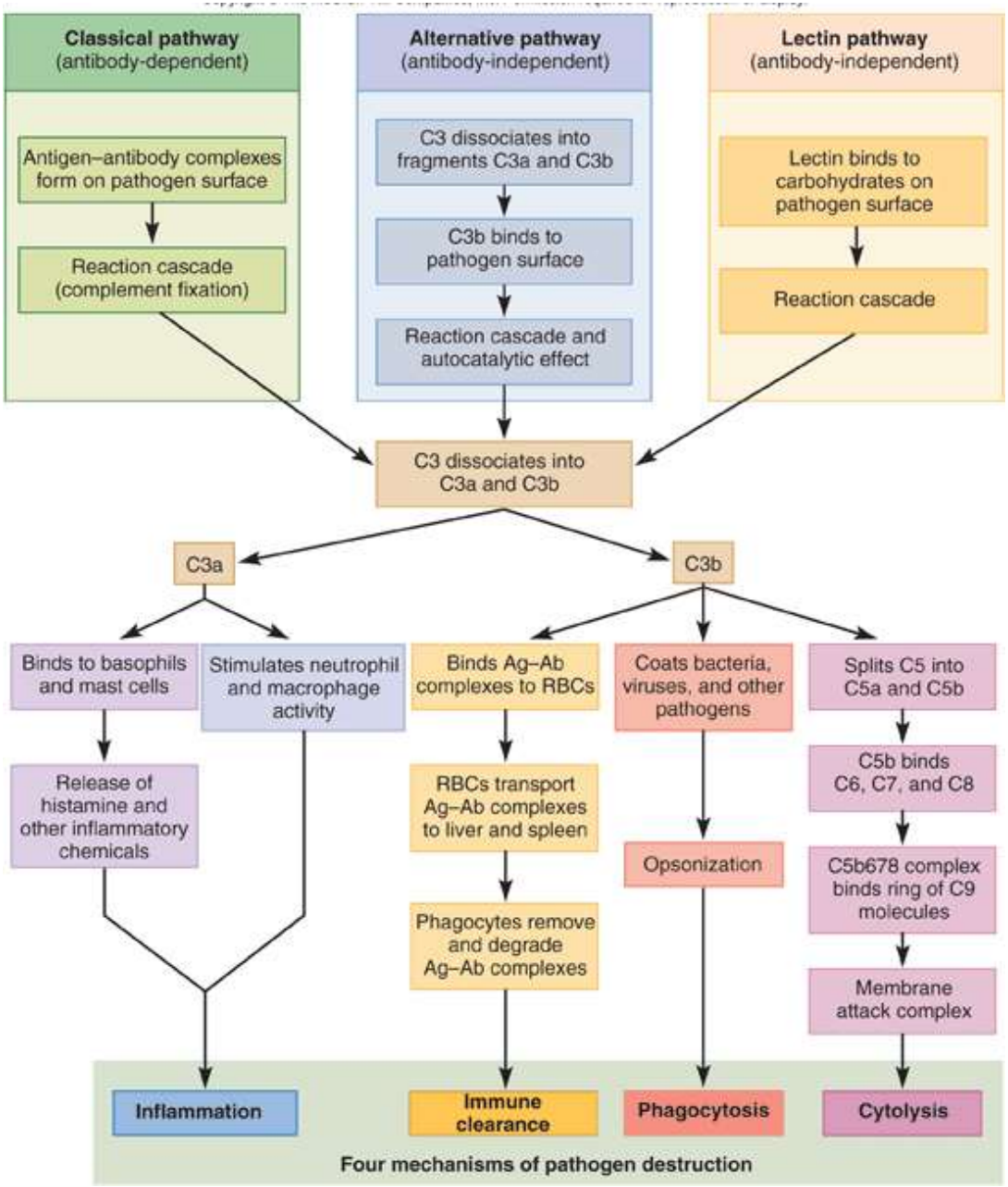
Interferon

- Secreted by certain cells infected by viruses
  - no benefit to the cell that secretes them // various cell types produce interferons (alpha, beta, gama) // including lymphocytes, macrophage, fibroblasts
  - alert neighboring cells and protect them from becoming infected
  - bind to surface receptors on neighboring cells /// activate second-messenger systems within
- Mechanism of actions:
  - alerted host cell metabolism to synthesizes various proteins that defend it from infection
    - breaks down viral genes
    - helps prevent replication of virus by “host cell”
  - activates NK cells and macrophages /// destroy infected cell before they can liberate a swarm of newly replicated viruses /// activated NK cells to destroy malignant cells

Complement System

- A group of 30 or more globular proteins that make powerful contributions to both / play important roll in both
  - nonspecific resistance
  - specific immunity
- synthesized mainly by the liver
- circulate in the blood inactive
- activated by presence of the pathogen
- **C3 is the "key" starter protein in the complement system**
  - C3 must be split into C3a and C3b to activate system
  - C3a and C3b activate separate "mechanisms"
  - three different "mechanisms" may activate complement by splitting C3 into C3a and C3b.

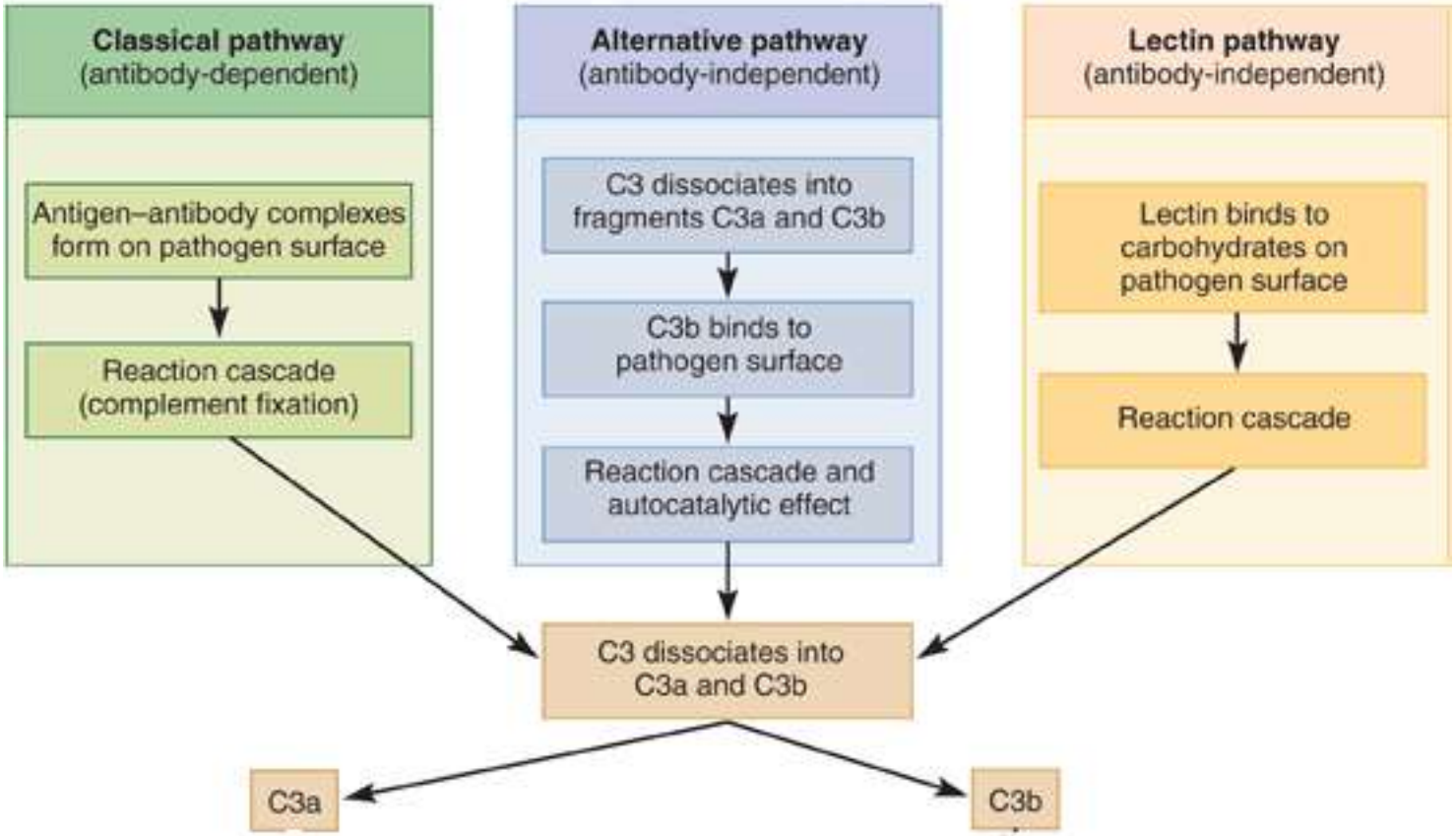
Complement Activation



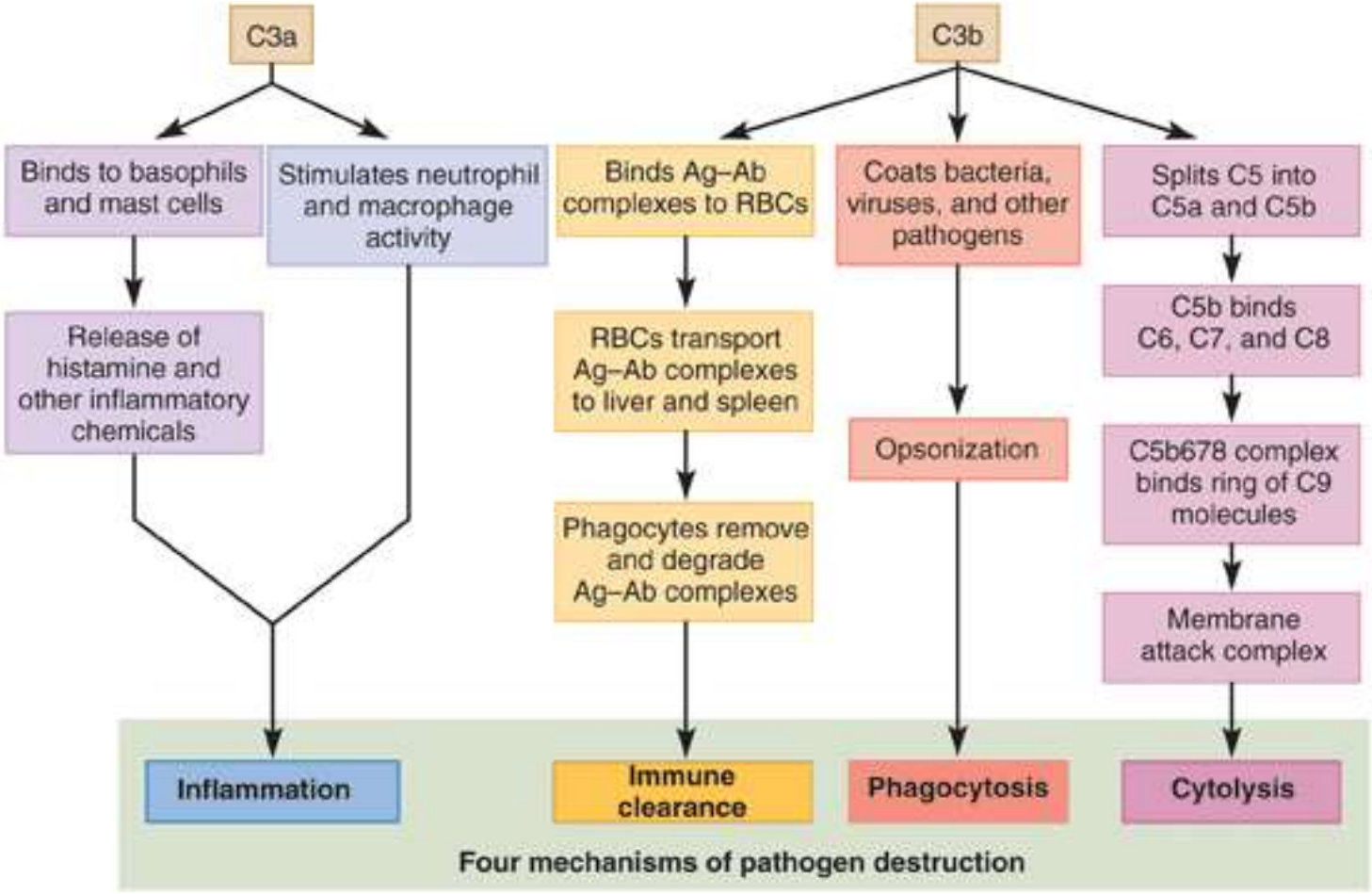
This is how complement is activated. (Three Options)

This pathway is part of specific immunity because it depends on the B Cells / plasma cells antibodies.

These pathways are part of the non specific resistance because they function independent of the B and T cells.



Complement's outcomes are a mixture of non-specific resistance and immunity:



Note: The following slides will examine the four outcomes of complement.

First of Four Outcomes of Complement
Immune Clearance

- C3b binds together antigen-antibody complexes with red blood cells
- these RBCs (with attached antigen-antibody) circulate through the liver and spleen
- macrophages of those organs strip off and destroy the Ag-Ab complexes leaving RBCs unharmed
- principal means of clearing foreign antigens from the bloodstream

Second of Four Outcomes of Complement
Phagocytosis

- neutrophils and macrophages **cannot phagocytize "naked"** bacteria, viruses, or other pathogens

- C3b assist them by **opsonization**

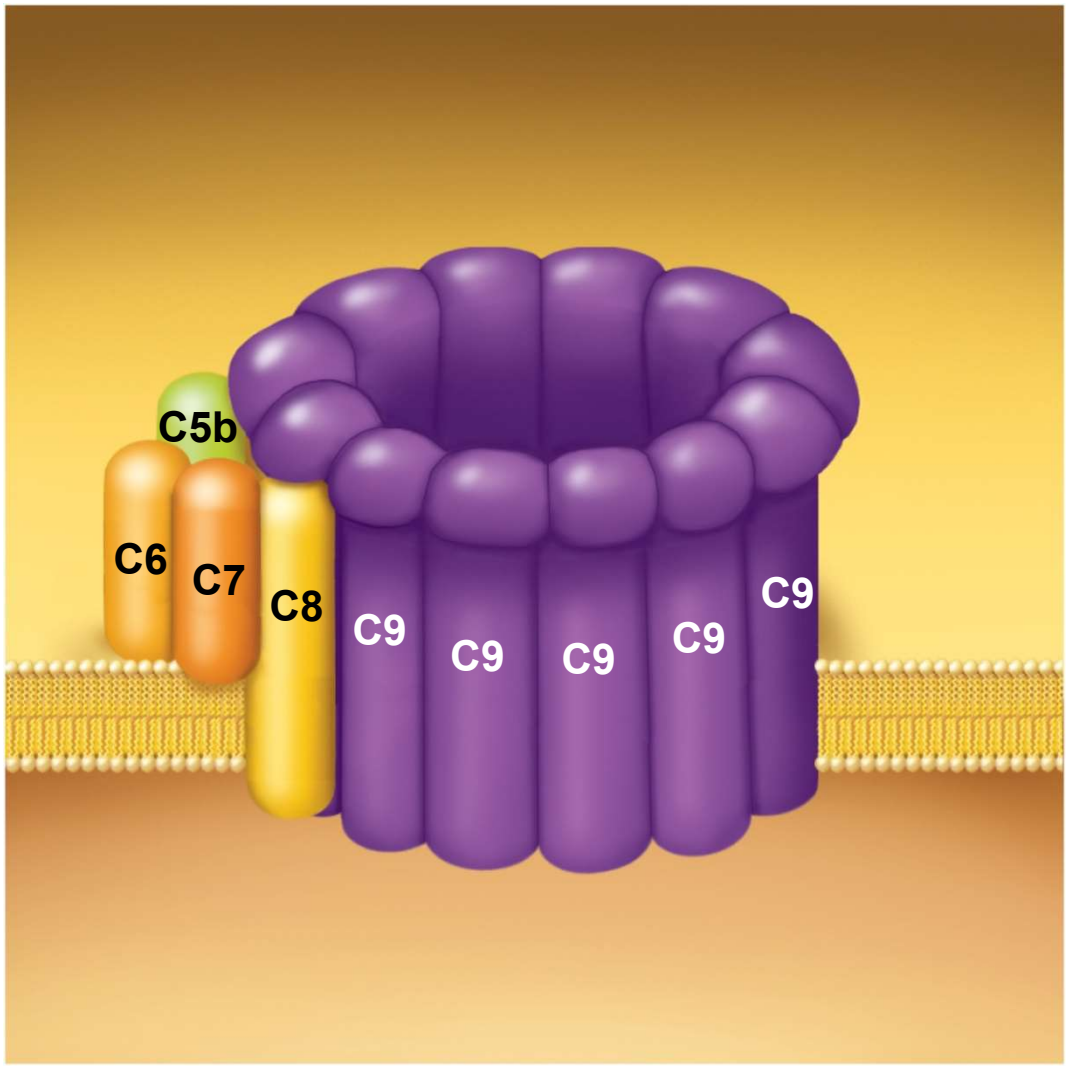
  – coats microbial cells and serves as binding sites for phagocyte attachment

  – makes the foreign cell more appetizing

Third of Four Outcomes of Complement
Cytolysis

- C3b splits other complement proteins
- bind to enemy cell
- attract more complement proteins (results in formation of the **membrane attack complex**)
  - forms a hole in the target cell
  - electrolytes leak out, water flows in rapidly, and **cell ruptures**

Membrane Attack Complex



Fourth Outcomes of Complement
Inflammation

- C3a stimulates mast cells and basophils to secrete histamine and other inflammatory chemicals / this "initiates" inflammation
- activates and attracts neutrophils and macrophages
- speed pathogen destruction in inflammation
- *Note: at the end of this presentation we will outline the individual steps of inflammation*

Inflammation
(Part of 2nd Line of Defense)

- Local defensive response to tissue injury of any kind, including trauma and infection

- general purposes of inflammation

  - limit spread of pathogens
  - destroy pathogens
  - remove debris from damaged tissue
  - initiate tissue repair

- four cardinal signs of inflammation

  - **redness**
  - **Swelling**
  - **Heat**
  - **pain**

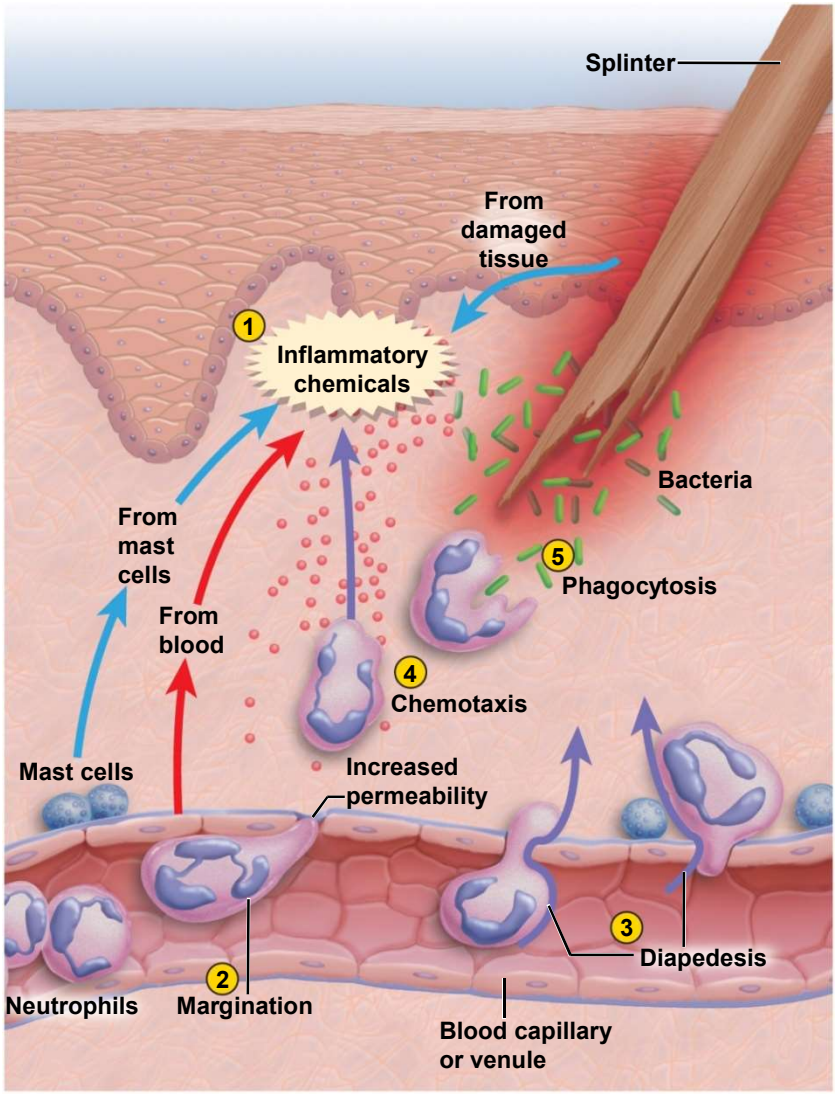
Inflammation – Four Cardinal Signs

- **heat** – results from hyperemia
- **redness** – due to hyperemia, and extravasated RBCs in the tissue
- **swelling** (edema) – due to increased fluid filtration from the capillaries
- **pain** – from direct injury to the nerves, pressure on the nerves from edema, stimulation of pain receptors by prostaglandins, bacterial toxins, and a kinin called **bradykinin**
- ***Note: immobilization of sore area like a joint is sometimes referred to as a "fifth event" but not a "cardinal sign"***

Four stages of inflammation

- Mobilization of body defenses
- Destruction and containment of pathogens
- Tissue cleanup
- Tissue repair

Inflammation - Mobilization of Defenses



Leukocyte behavior

- **Margination**
  - **selectins** cause leukocytes to adhere to blood vessel walls
- **Diapedesis** (emigration)
  - leukocytes squeeze between endothelial cells into tissue space

Inflammation - Mobilization of Defenses

- selectins – cell-adhesion molecules made by endothelial cells that aid in the recruitment of leukocytes
  - make membranes sticky and snag leukocytes
- margination – adhesion of the leukocytes to the vessel wall
- diapedesis or emigration - leukocytes crawl through gaps in the endothelial cells and enter tissue fluid
- extravasated – cells and chemicals that have left the bloodstream

Inflammation - Containment and Destruction of Pathogens

- a priority of inflammation is to prevent the pathogens from spreading throughout the body
  - fibrinogen that filters into tissue fluid clots /// forms a sticky mesh that walls off microbes
  - heparin prevents clotting at site of injury
    - pathogens are in a fluid pocket surrounded by clot
    - attacked by antibodies, phagocytes, and other defenses
- neutrophils, the chief enemy of bacteria, accumulate at the injury site within an hour /// after leaving the bloodstream, move to site of infection by chemotaxis

Inflammation - Containment and Destruction of Pathogens

- chemotaxis – attraction to chemicals such as bradykinin and leukotrienes that guide them to the injury site
- neutrophils are the “first responders” to arrive at site of infection
- kill bacteria by phagocytosis & respiratory burst (RB main killing force)
  - secrete cytokines for recruitment of macrophages, NK cells, and additional neutrophils
  - macrophages and T cells secrete colony-stimulating factor to stimulate leukopoiesis
    - neutrophilia – 5000 cells/μL to 25,000 cells/μL in bacterial infection
    - eosinophilia – elevated eosinophil count in allergy or parasitic infection

Inflammation - Tissue Cleanup

- Macrophage are the primary agents of tissue cleanup and repair
  - arrive in 8 to 12 hours
  - as monocytes in blood emigrate into tissue spaces they become macrophage
    - engulf and destroy bacteria
    - engulf damaged host cells
    - engulf dead and dying neutrophils
    - Remember, macrophage are also APC

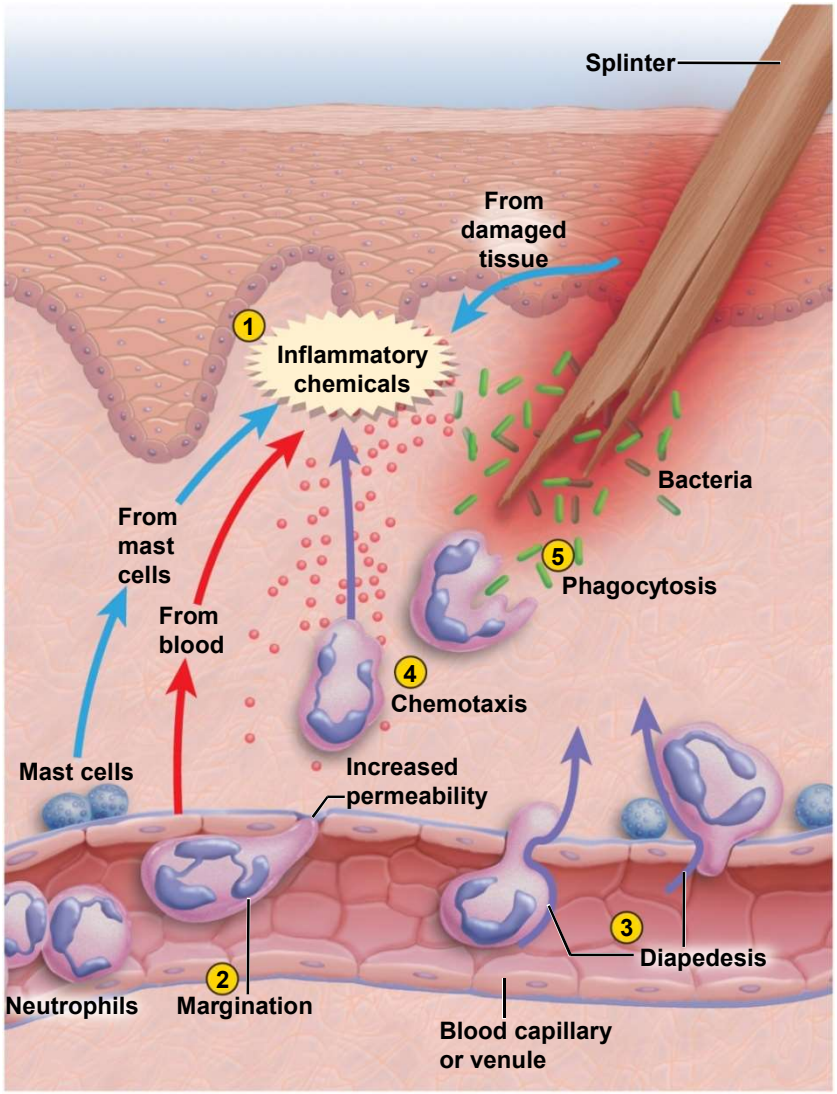
Inflammation - Tissue Cleanup

- edema contributes to tissue cleanup
  - swelling compresses veins and reduces venous drainage
  - this forces open lymphatic capillaries to promote interstitial fluid inot lymphatic vessels
  - lymphatics collect and move lymh into lymph nodes /// remove bacteria, dead cells, proteins, and tissue debris better than blood capillaries
- pus – accumulation of dead neutrophils, bacteria, other cellular debris, and tissue fluid form a pool of yellowish fluid
- abscess – accumulation of pus in tissue surrounded by fibrin

Inflammation - Tissue Repair

- **platelet-derived growth factor** secreted by blood platelets and endothelial cells in injured area
  - stimulates fibroblasts to multiply
  - synthesize new collagen fibers
- **hyperemia** delivers oxygen, amino acids, and other necessities for protein synthesis
- increased **heat** increases metabolic rate, speeds mitosis, and tissue repair
- **fibrin clot** forms a scaffold for tissue reconstruction
- **pain** makes us limit the use of a body part so it has a chance to rest and heal.

Inflammation - Mobilization of Defenses



Leukocyte behavior

- **Margination**
  - **selectins** cause leukocytes to adhere to blood vessel walls
- **Diapedesis** (emigration)
  - leukocytes squeeze between endothelial cells into tissue space

What is fever?

- An abnormally elevation of body temperature
  - Also called pyrexia /// febrile refers to pyrexia
  - results from trauma, infections, drug reactions, brain tumors, and other causes // e.g. inflammation
- fever is an adaptive defense mechanism /// a low fever will do more good than harm
  - promotes interferon activity
  - elevates metabolic rate and accelerates tissue repair
  - inhibits reproduction of bacteria and viruses /// limits iron to bacteria
- **antipyretic** – fever-reducing medications by inhibiting PGE_2

What causes fever?

- initiation of fever by exogenous pyrogens
  - fever producing agents
  - glycolipids on bacterial and viral surfaces
- attacking neutrophils and macrophages secrete chemicals like interleukins, interferons, and others that act as endogenous pyrogens
  - stimulate neurons in the anterior hypothalamus to secrete prostaglandin E_2
  - PGE_2 raises hypothalamic set point for body temperature
- stages of fever = onset, stadium, defervescence

Inflammation – Course of Fever