BME – 220
Biomaterials

Abeer Syed
Department of Biomedical Engineering
King Faisal University

Biological Interaction of Biomaterials - Biocompatibility and immune response

Unit 7

Host Responses to Biomaterials

Implantation of a biomaterial initiates the **inflammatory** response:

- response of vascularized tissue to local injury
- severity indicates biocompatibility of material

Cooperative Signaling Cascades:

1. Coagulation Cascade
2. Complement Alternative Pathway

} initiated by adsorbed proteins

The **complement** is a component of the immune system.
Immune system function: to protect against pathogens

Immune System

Innate (Native) Immunity

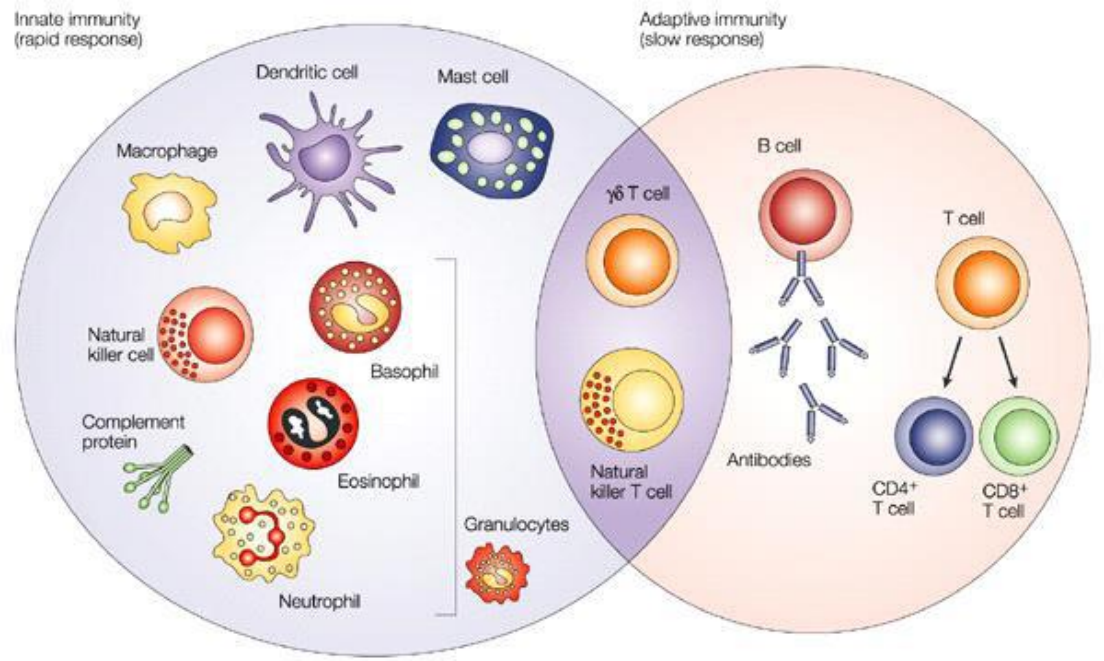
- first line of defense
- nonspecific response to invading pathogens
- elicits adaptive response

- **Physical/chemical barriers:** epithelia, antimicrobial proteins
- **Blood proteins:** complement; cytokines (regulatory)
- **Cells:** phagocytes (macrophages, neutrophils), natural killer cells

Adaptive (Acquired) Immunity

- specificity to distinct foreign biomolecules (antigens)
- memory of exposure

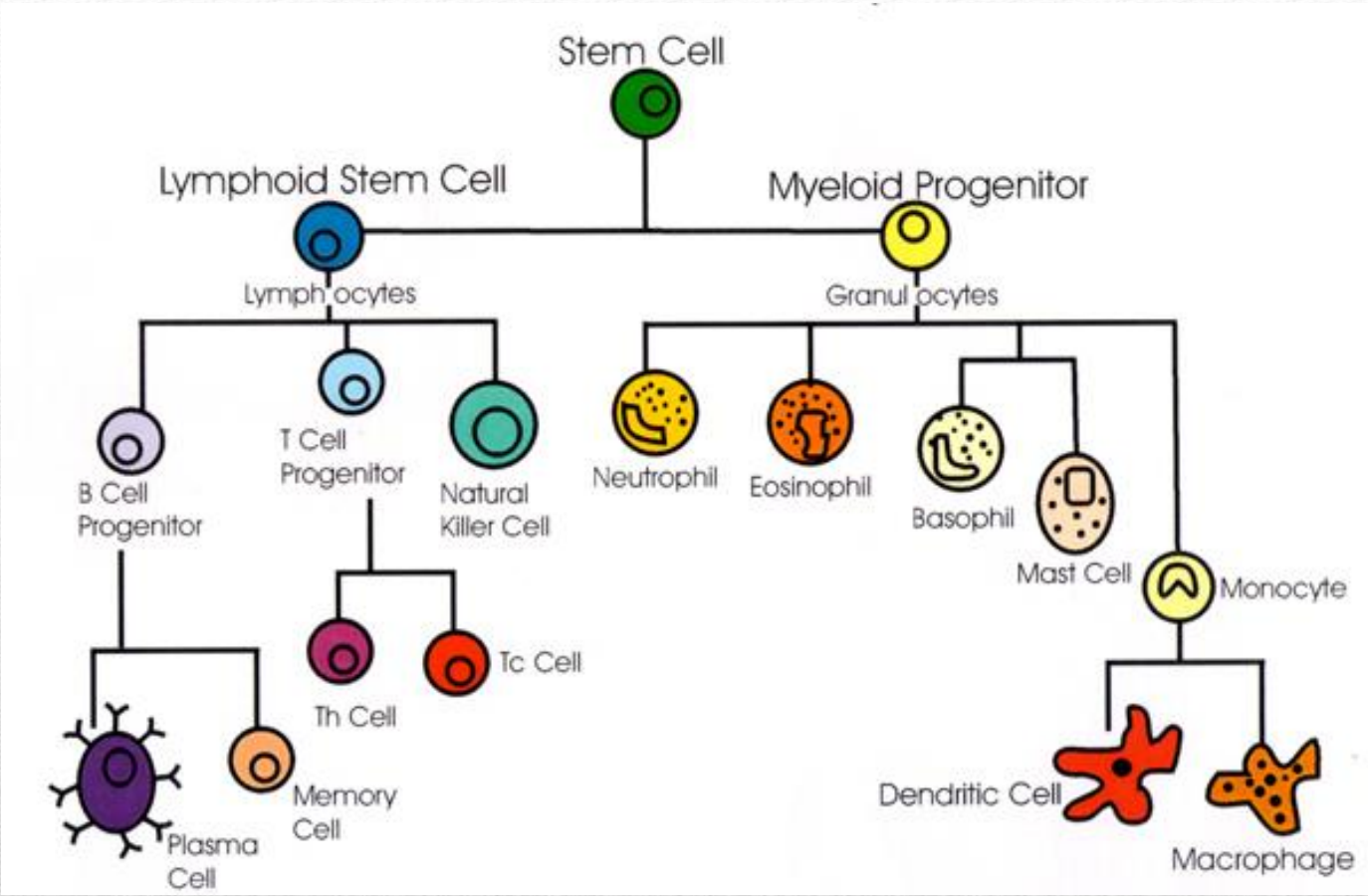
- **Blood proteins:** antibodies (immunoglobulins), cytokines
- **Cells:** lymphocytes (T cells, B cells)



Vertebrate Immunity		
Innate Immune System		Adaptive Immune System
Physical Barriers	Internal Defenses	
• Skin, hair, cilia • Mucus membranes • Mucus and chemical secretions • Digestive enzymes in mouth • Stomach acid	• Inflammatory response • Complement proteins • Phagocytic cells • Natural killer (NK) cells	• Antibodies and the humoral immune response • Cell-mediated immune response • Memory response

Immune System

Nature Reviews Immunology, 5, 101-111, 2005.



Cells of Immune System

Cells of Immune System

- **Phagocytes, or Phagocytic cells**: Phagocyte means “eating cell”, which describes what role phagocytes play in the immune response. Phagocytes circulate throughout the body, looking for potential threats, like bacteria and viruses, to engulf and destroy. You can think of phagocytes as security guards on patrol.
- **Macrophages:** Macrophages are efficient phagocytic cells that can leave the circulatory system by moving across the walls of capillary vessels. The ability to roam outside of the circulatory system is important, because it allows macrophages to hunt pathogens with less limits. Macrophages can also release cytokines in order to signal and recruit other cells to an area with pathogens.

Cells of Immune System

- **Mast cells:** Mast cells are found in mucous membranes and connective tissues, and are important for wound healing and defense against pathogens via the inflammatory response. When mast cells are activated, they release cytokines and granules that contain chemical molecules to create an **inflammatory cascade**. Mediators, such as histamine, cause blood vessels to dilate, increasing blood flow and cell trafficking to the area of infection. The cytokines released during this process act as a messenger service, alerting other immune cells, like neutrophils and macrophages, to make their way to the area of infection, or to be on alert for circulating threats.

Cells of Immune System

- **Neutrophils:** Neutrophils are phagocytic cells that are also classified as **granulocytes** because they contain granules in their cytoplasm. These granules are very toxic to bacteria and fungi, and cause them to stop proliferating or die on contact.
- The bone marrow of an average healthy adult makes approximately 100 billion new neutrophils per day. Neutrophils are typically the first cells to arrive at the site of an infection because there are so many of them in circulation at any given time.

Cells of Immune System

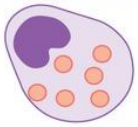
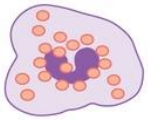
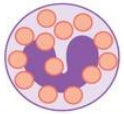
- **Eosinophils:** Eosinophils are granulocytes that target multicellular parasites. Eosinophils secrete a range of highly toxic proteins and free radicals that kill bacteria and parasites. The use of toxic proteins and free radicals also causes tissue damage during allergic reactions, so activation and toxin release by eosinophils is highly regulated to prevent any unnecessary tissue damage.
- While eosinophils only make up 1-6% of the white blood cells, they are found in many locations, including the thymus, lower gastrointestinal tract, ovaries, uterus, spleen, and lymph nodes.

Cells of Immune System

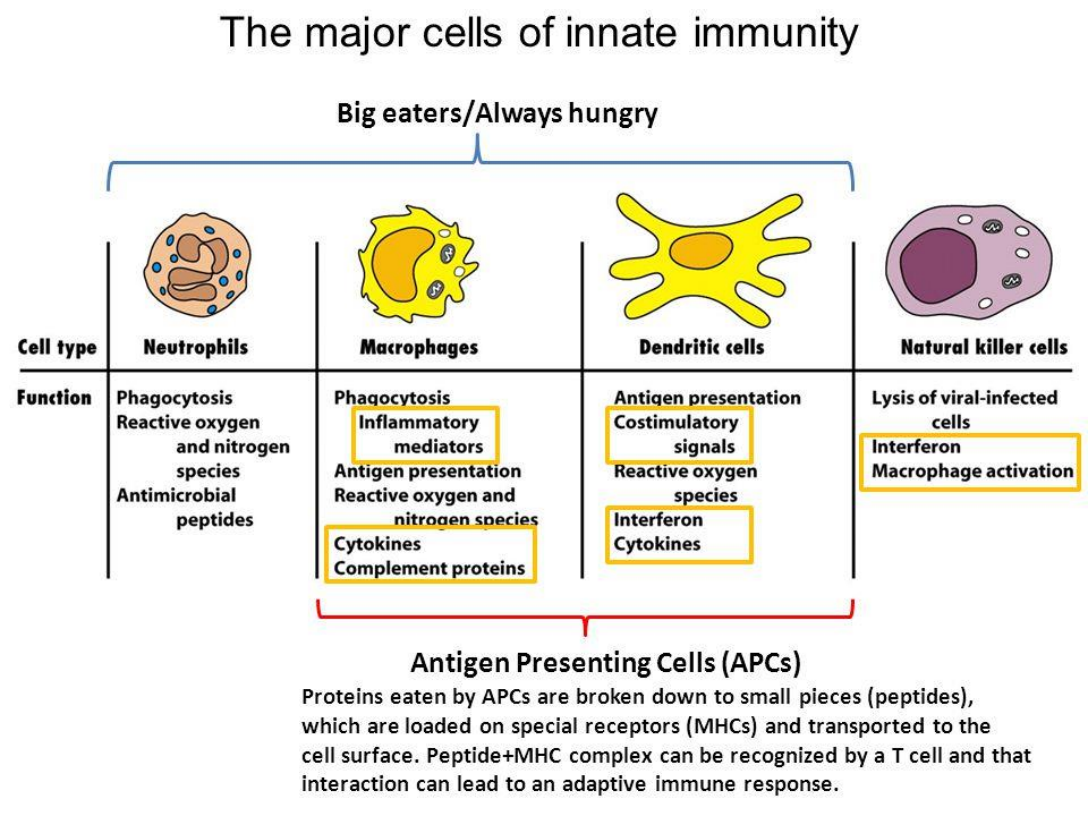
- **Basophils:** Basophils are also granulocytes that attack multicellular parasites. Basophils release histamine, much like mast cells. The use of histamine makes basophils and mast cells key players in mounting an allergic response.
- **Natural Killer cells:** Natural Killer cells (NK cells), do not attack pathogens directly. Instead, natural killer cells destroy infected host cells in order to stop the spread of an infection. Infected or compromised host cells can signal natural kill cells for destruction through the expression of specific receptors and antigen presentation.

Cells of Immune System

- **Dendritic cells:** Dendritic cells are **antigen-presenting cells** that are located in tissues, and can contact external environments through the skin, the inner mucosal lining of the nose, lungs, stomach, and intestines. Since dendritic cells are located in tissues that are common points for initial infection, they can identify threats and act as messengers for the rest of the immune system by antigen presentation. Dendritic cells also act as bridge between the innate immune system and the adaptive immune system.

Cell type	Characteristics	Location	Image
Mast cell	Dilates blood vessels and induces inflammation through release of histamines and heparin. Recruits macrophages and neutrophils. Involved in wound healing and defense against pathogens but can also be responsible for allergic reactions.	Connective tissues, mucous membranes	
Macrophage	Phagocytic cell that consumes foreign pathogens and cancer cells. Stimulates response of other immune cells.	Migrates from blood vessels into tissues.	
Natural killer cell	Kills tumor cells and virus-infected cells.	Circulates in blood and migrates into tissues.	
Dendritic cell	Presents antigens on its surface, thereby triggering adaptive immunity.	Present in epithelial tissue, including skin, lung and tissues of the digestive tract. Migrates to lymph nodes upon activation.	
Monocyte	Differentiates into macrophages and dendritic cells in response to inflammation.	Stored in spleen, moves through blood vessels to infected tissues.	
Neutrophil	First responders at the site of infection or trauma, this abundant phagocytic cell represents 50-60 percent of all leukocytes. Releases toxins that kill or inhibit bacteria and fungi and recruits other immune cells to the site of infection.	Migrates from blood vessels into tissues.	
Basophil	Responsible for defense against parasites. Releases histamines that cause inflammation and may be responsible for allergic reactions.	Circulates in blood and migrates to tissues.	
Eosinophil	Releases toxins that kill bacteria and parasites but also causes tissue damage.	Circulates in blood and migrates to tissues.	

Cells of Immune System



Cells of Immune System

Complement System

- **Complement system:** an array of more than 30 proteins that mediate immune response.
  - discriminates “foreign” from “self” through adsorbed proteins/ protein fragments (C3b, C4b)
  - recruit and activate phagocytes (C3a, C5a)
  - lysis of pathogens via membrane pore formation (C5b, C6-C9)
- 11 are named using alphabets, B, C1- 9 and D.

- **The Complement System**
- The complement system (also called the **complement cascade**) is a mechanism that **complements** other aspects of the immune response. Typically, the complement system acts as a part of the innate immune system, but it can work with the adaptive immune system if necessary.
- The complement system is made of a variety of proteins that, when inactive, circulate in the blood. When activated, these proteins come together to initiate the complement cascade, which starts the following steps:

Complement System

https://www.khanacademy.org/test-prep/mcat/organ-systems/the-immune-system/a/innate-immunity

- **Opsonization:** Opsonization is a process in which foreign particles are marked for phagocytosis. All of the pathways require an antigen to signal that there is a threat present. Opsonization tags infected cells and identifies circulating pathogens expressing the same antigens.
- **Chemotaxis:** Chemotaxis is the attraction and movement of macrophages to a chemical signal. Chemotaxis uses cytokines and chemokines to attract macrophages and neutrophils to the site of infection, ensuring that pathogens in the area will be destroyed. By bringing immune cells to an area with identified pathogens, it improves the likelihood that the threats will be destroyed and the infection will be treated.

Complement System

https://www.khanacademy.org/test-prep/mcat/organ-systems/the-immune-system/a/innate-immunity

- **Cell Lysis:** Lysis is the breaking down or destruction of the membrane of a cell. The proteins of the complement system puncture the membranes of foreign cells, destroying the integrity of the pathogen. Destroying the membrane of foreign cells or pathogens weakens their ability to proliferate, and helps to stop the spread of infection.
- **Agglutination:** Agglutination uses antibodies to cluster and bind pathogens together. By bringing as many pathogens together in the same area, the cells of the immune system can mount an attack and weaken the infection. Other innate immune system cells continue to circulate throughout the body in order to track down any other pathogens that have not been clustered and bound for destruction.

Complement System

https://www.khanacademy.org/test-prep/mcat/organ-systems/the-immune-system/a/innate-immunity

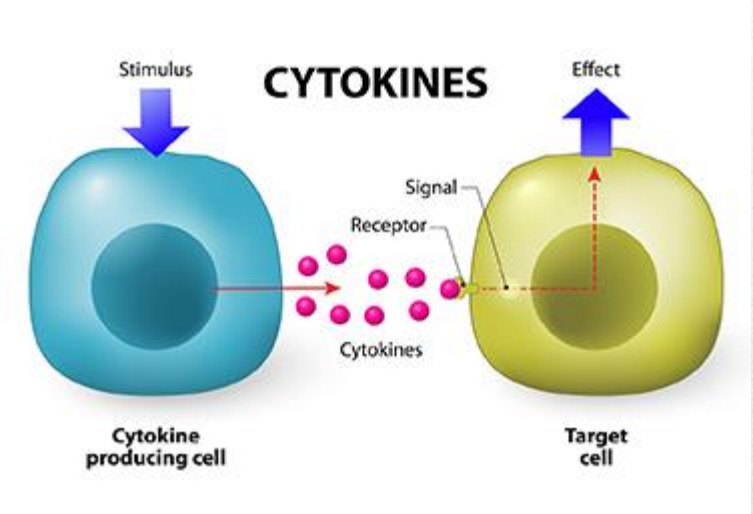
- The steps of the complement cascade facilitate the search for and removal of antigens by placing them in large clumps, making it easier for other aspects of the immune system to do their jobs. Remember that the complement system is a supplemental cascade of proteins that assists, or “complements” the other aspects of the innate immune system.
- The innate immune system works to fight off pathogens before they can start an active infection. For some cases, the innate immune response is not enough, or the pathogen is able to exploit the innate immune response for a way into the host cells. In such situations, the innate immune system works with the adaptive immune system to reduce the severity of infection, and to fight off any additional invaders while the adaptive immune system is busy destroying the initial infection.

Complement System

https://www.khanacademy.org/test-prep/mcat/organ-systems/the-immune-system/a/innate-immunity

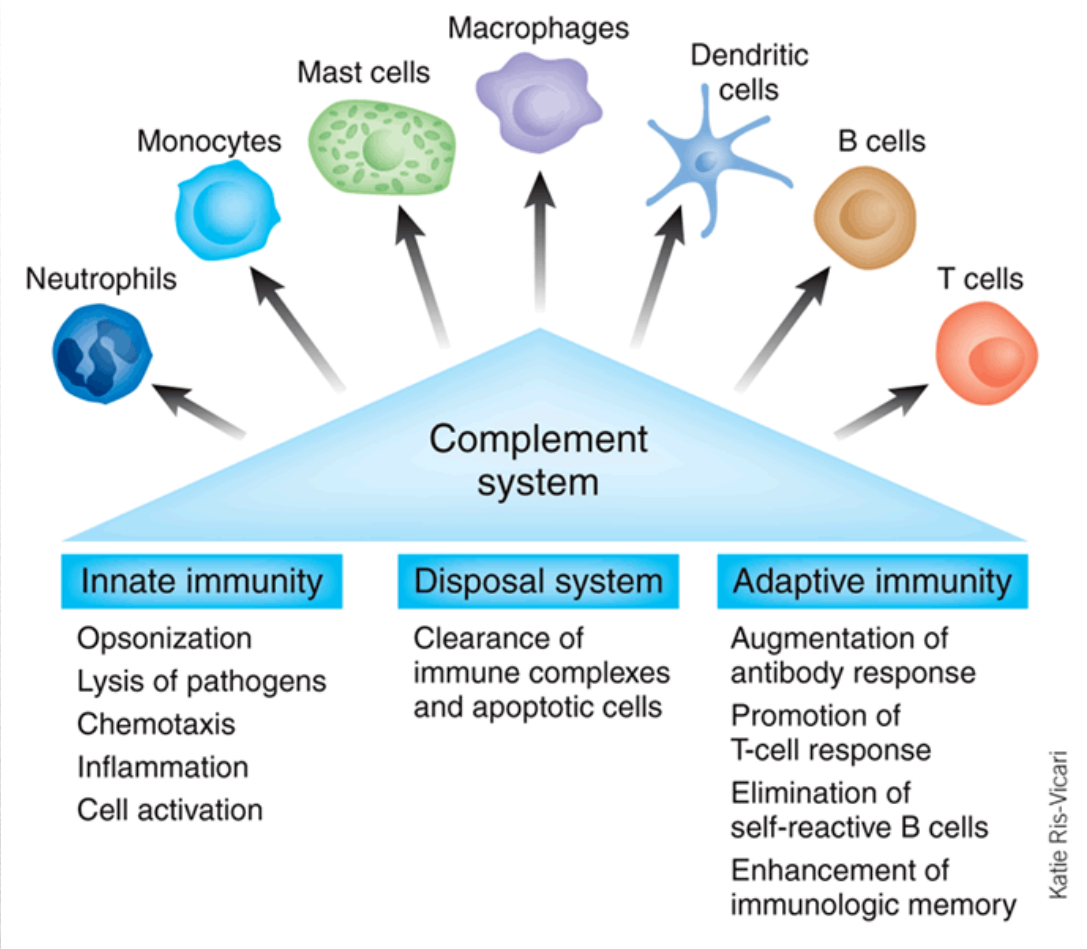
Cytokines

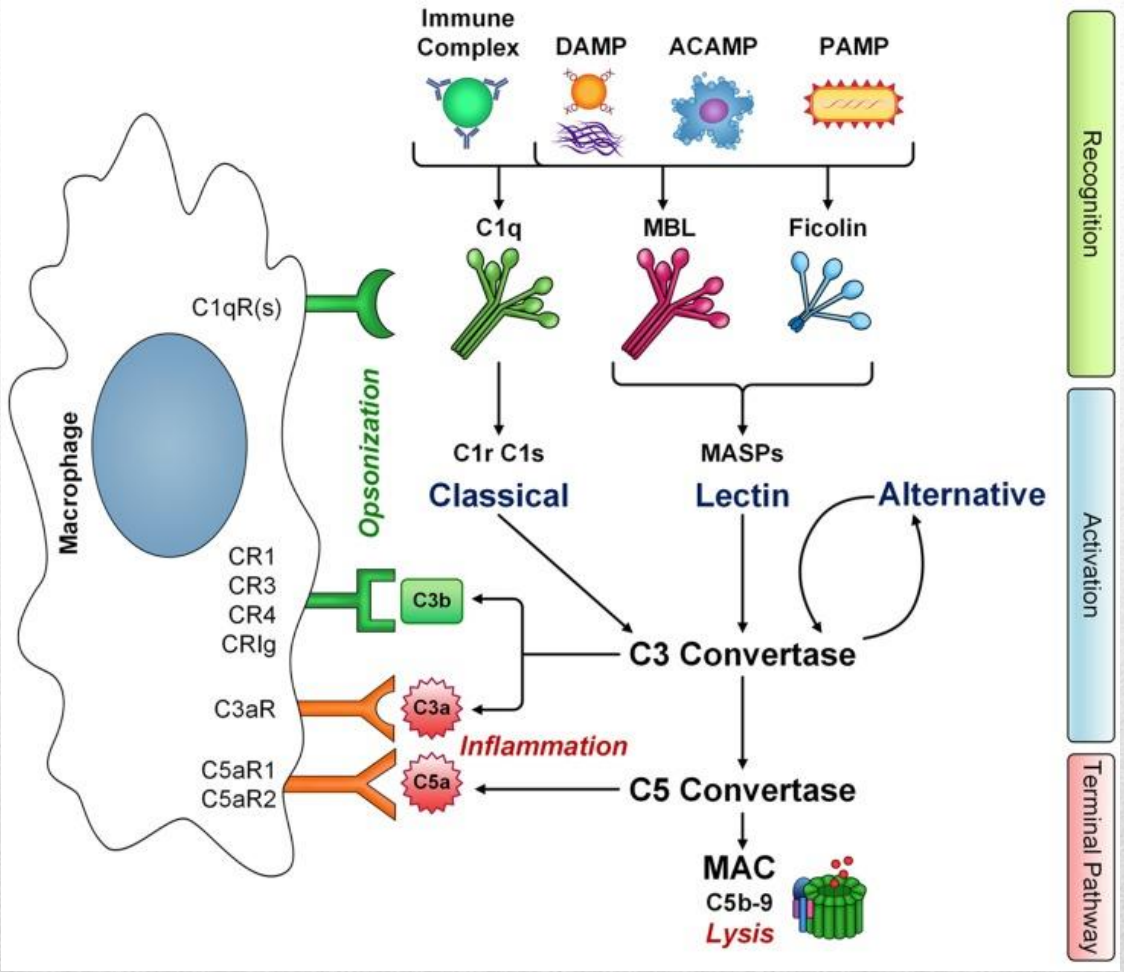
- Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells.
- Chemokines, the name being an abbreviation of the term **chemotactic cytokines,** are protein mediators that cause the migration of various cell types, usually toward the source or highest concentration of the stimulus.



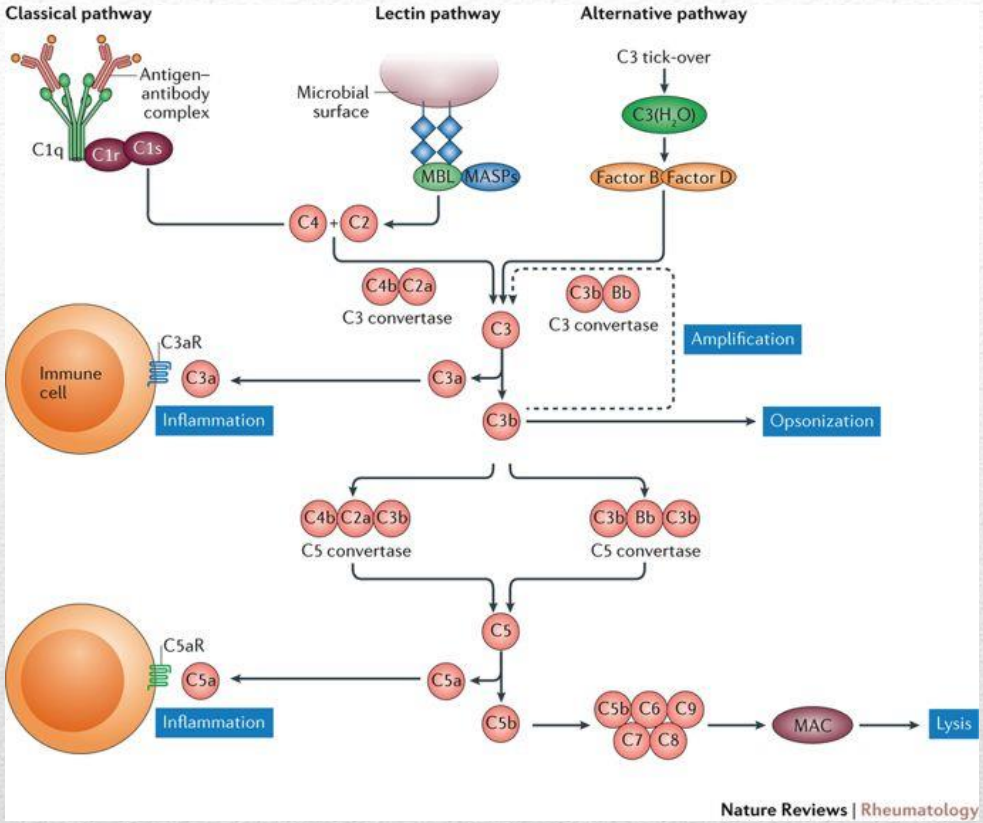
Complement System

- The complement system as a bridge between innate and adaptive immunity.

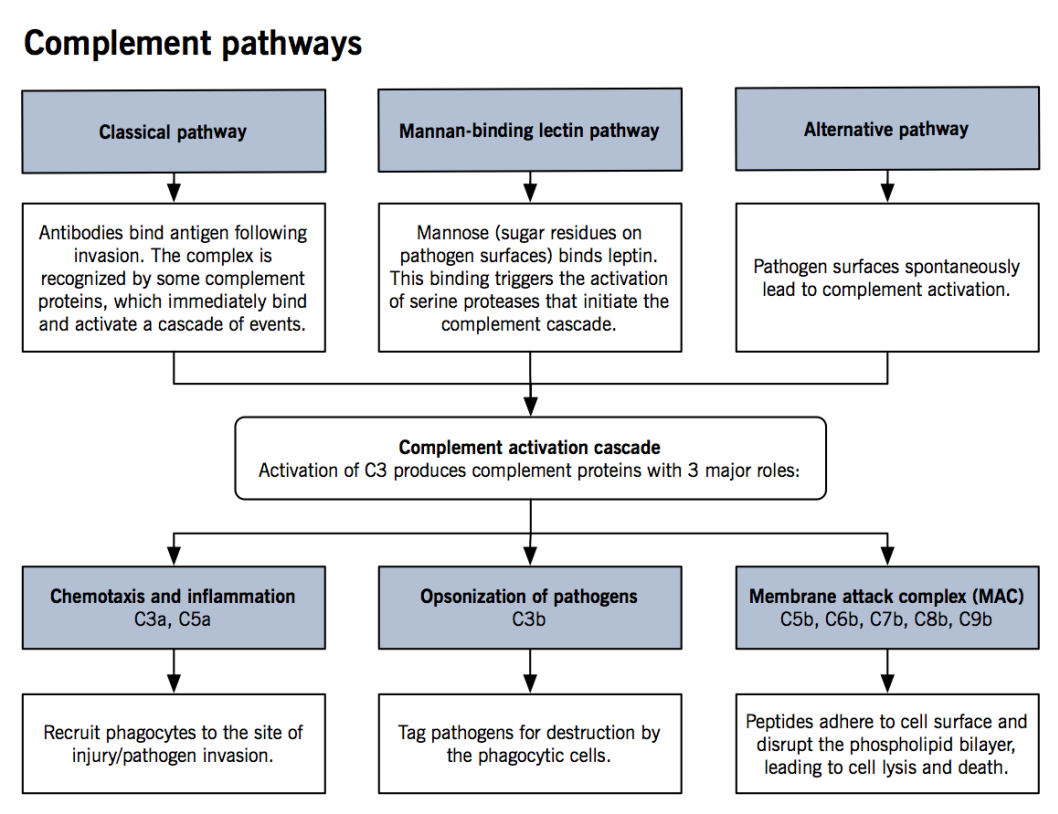




Complement Pathways



Complement Pathways

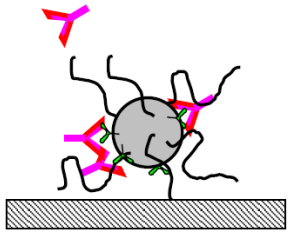


Complement Pathways

3 recognized pathways (to C5 convertase)

Classical pathway:

antigen-antibody immune complex (IC)



binds and activates C1 (autocatalytic proteolysis) initiating an enzymatic cascade

C1 → C1s
C4 → C4b
C2 → C2b
C3 → C3b
C5 → C5a/C5b

soluble fragment (16 kDa): recruits phagocytes by chemotaxis

insoluble fragment (170 kDa): initiates membrane attack complex (MAC) C5b•C6•C7•C8•C9

MAC pore formation compromises bacterial cell membrane

Complement Pathways

Lectin pathway (discovered in 1990's):

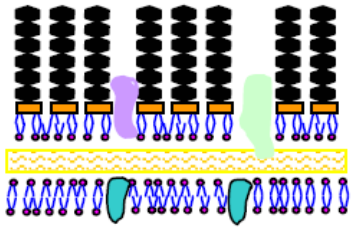
mannan binding lectin (MBL)
binds carbohydrates on pathogen

MBL-associated serine proteases (MASP-1, -2)
complexes with MBL

activated MASP's cleave C4 → C4b

remaining cascade follows classical pathway

bacteria cell wall
(gram negative)



Complement Pathways

Complement Pathways

Alternative pathway:

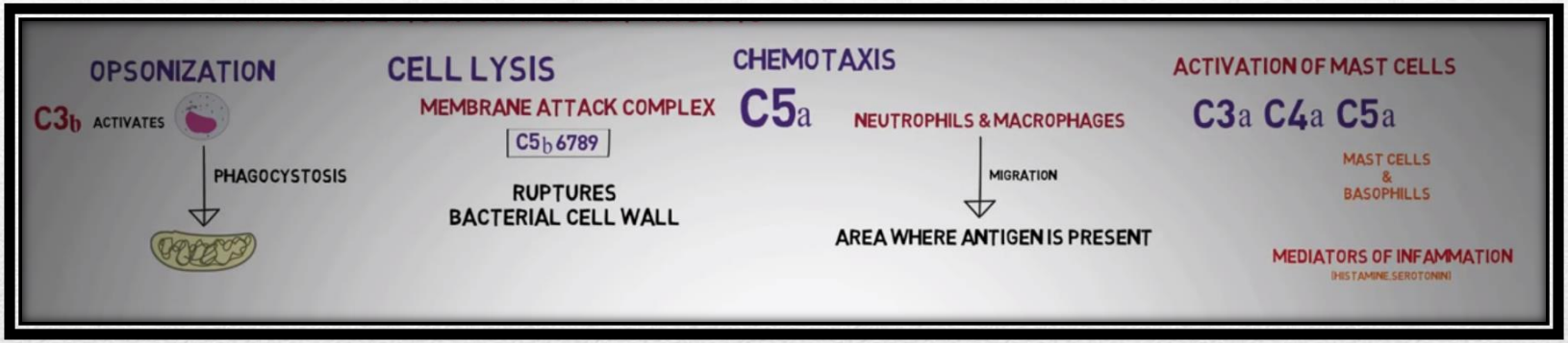
nonselective pathway of complement (any foreign surface)

C3 → C3b occurs continuously in plasma at low frequency

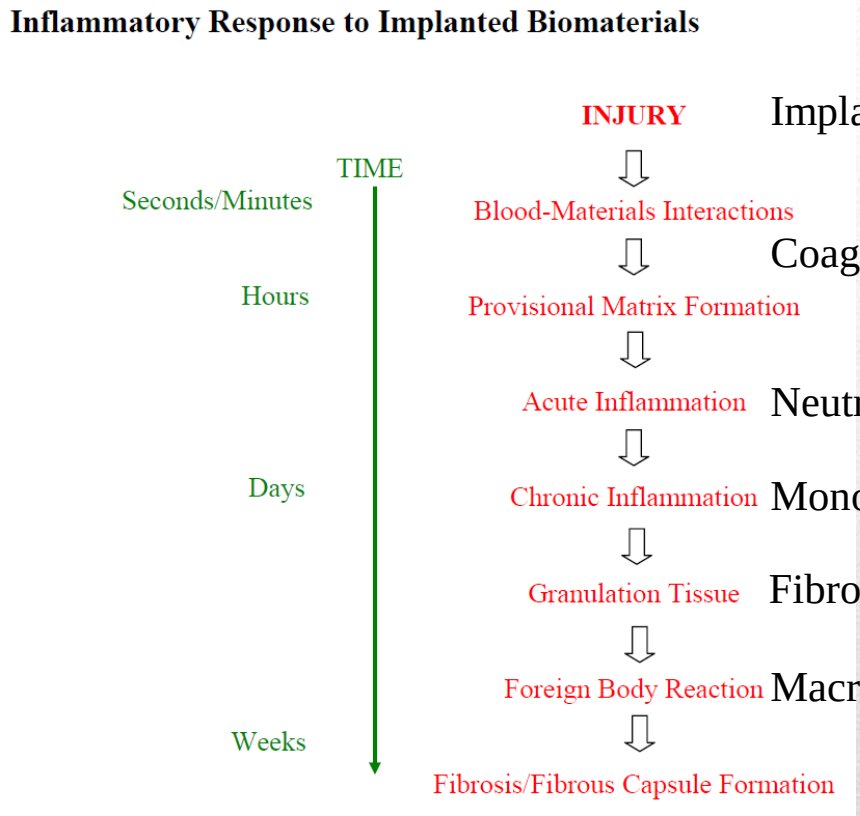
C3b adsorbs on foreign surfaces (biomaterial)

cofactor B → C3b•Bb complex
amplifies C3 → C3b
C3b•C3b•Bb complex
C5 → C5a/C5b

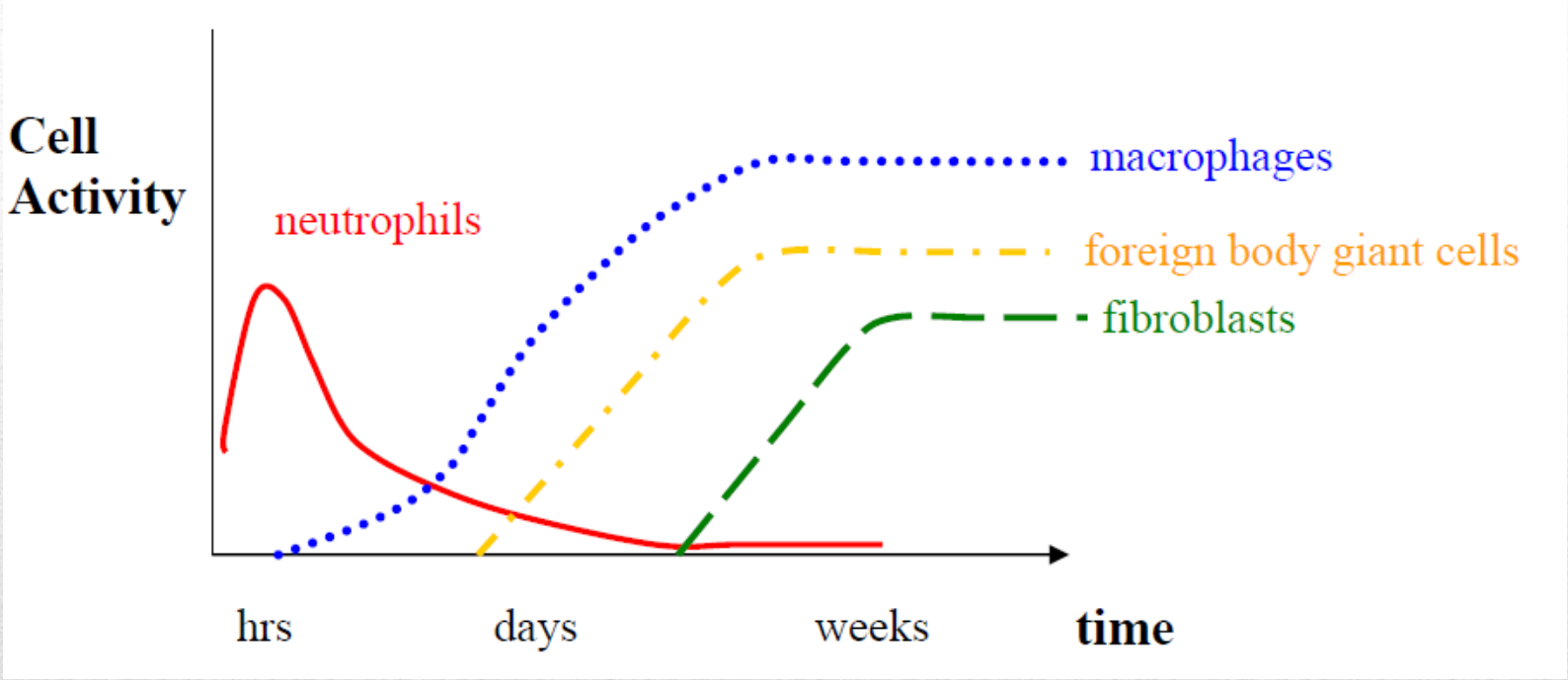
Soluble complement protein fragments C3a and C5a recruit phagocytes to site of injury



Effects of Complement Products



Host Responses to Biomaterials



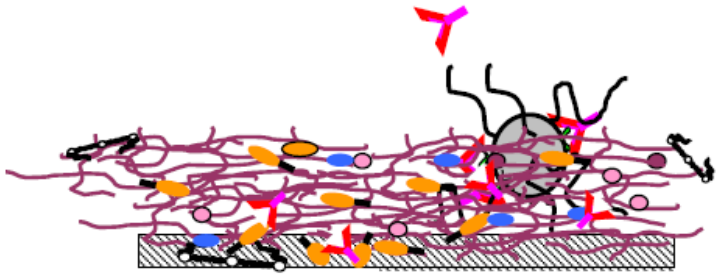
Host Responses to Biomaterials

Neutrophils

Associated with acute inflammatory response (minutes→1-2 days)

- "first responders" 3-5M/ml (short-lived)
- bind C3a/C5a via complement receptors (CR's)
- chemotactic to C5a: migrate to inflammation site
- On site, neutrophils bind to C3b, catalyzing release of cytotoxic species: H_2O_2, O^{2-} (superoxide radical) OH, enzymes ⇒ attack/engulf/degrade invading microbes

Released products from neutrophils, activated platelets and endothelial cells, along with fibrin, form the **provisional matrix**



- scaffold for cell attachment

- sustained release of signaling molecules

Monocytes

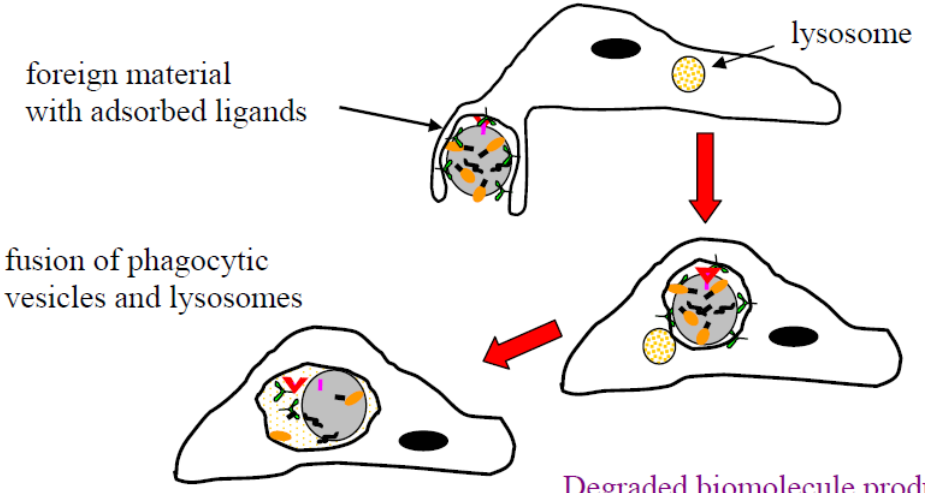
- Bind C3a/C5a ⇒ follow the course of neutrophils
- Evolve to macrophages
- Associated with chronic inflammation

  days→ weeks/months (or even a lifetime)
- On site, macrophages bind C3b, secrete reactive species, enzymes, cytokines (immune cell regulators, ex. IL-1), fibronectin, growth factors (ex. fibroblast growth factor, epidermal growth factor), coagulation factors

Macrophage

Macrophage response depends on foreign material properties…

- fluids or small particles (micron-sized)
  → engulfed & degraded "phagocytosis"



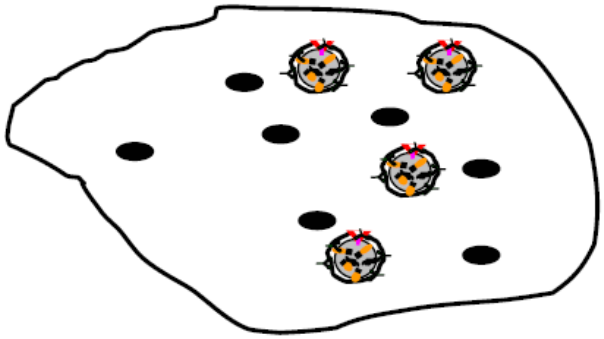
Degraded biomolecule products:
amino acids, sugars, lipids

Nondegradable products accumulate

Foreign Body Giant Cells

- Numerous particulate debris or materials with high roughness

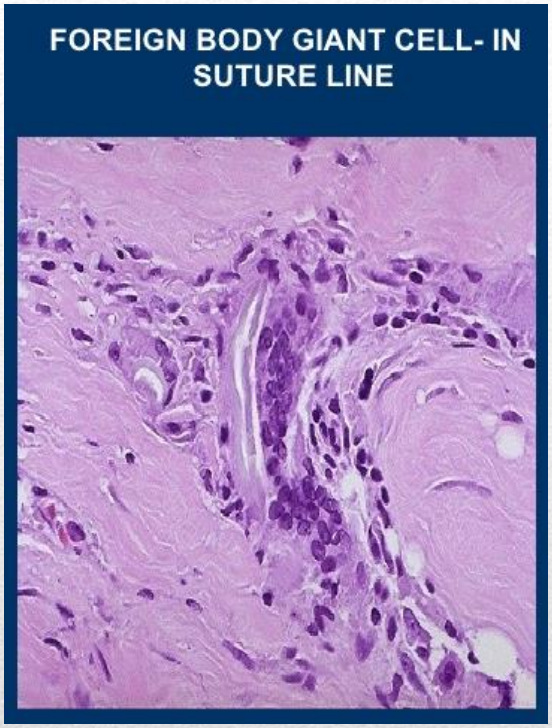
  → fusion of macrophages into multinuclear foreign body giant cells (FBGCs)



- smooth, inert implants

  FBGCs absent (nothing to engulf)

  → macrophage layer surrounds implant



Fibroblasts

Macrophage/FBGC products (FN, FGF) recruit fibroblasts

Fibroblasts (connective tissue cells)

- deposit collagen

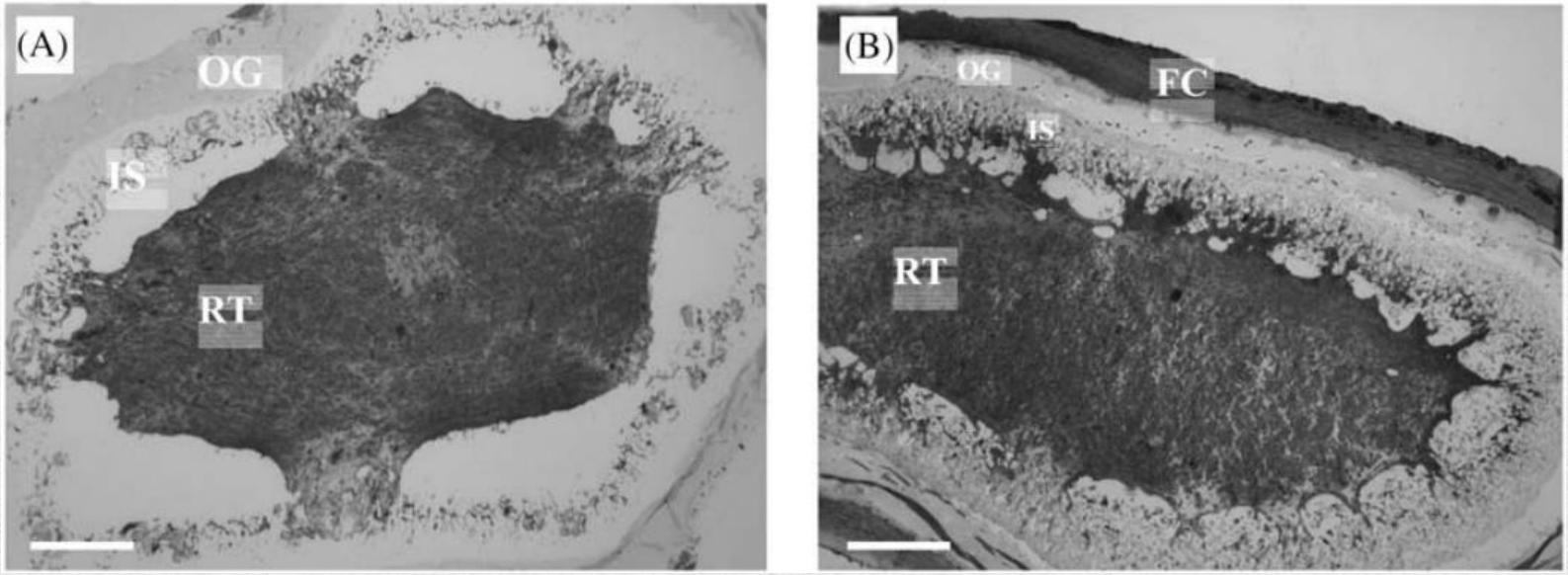
  → pink "granulation tissue" (appears in 3-5 days)

- accompanied by capillary sprouting (angiogenesis)

Wound healing histology: foreign body reaction

presence of FBGCs/macrophages, granulation tissue, capillaries at tissue/material interface

- Connective tissue remodeling ⇒ thin, encapsulating fibrous layer (fibrosis) isolates implant and foreign body rxn (weeks)



(A) Low-power photomicrograph of a representative 8 week nerve conduit tube in cross-section, comprised of Inner Spongy (IS) and Outer Gel (OG) layers, with its contained Regenerating Tissue (RT). (B) Low-power photomicrograph of a tube at 16 weeks with a contained RT and IS and OG layers. The tube was enveloped by a vascularized fibrous capsule (FC).

Wound Healing

J.S. Belkas et al., *Biomaterials* **26** (2005) 1741.

Wound Healing

- Formation of scar tissue vs. parenchymal tissue (tissue of specialized function) depends on:
  - extent of parenchymal tissue damage (esp. tissue framework)
  - parenchymal cell proliferation capacity

- Parenchyma/Stroma: The parenchyma of an organ consists of that tissue which conducts the specific function of the organ and which usually comprises the bulk of the organ. Stroma is everything else -- connective tissue, blood vessels, nerves, ducts. The parenchyma/stroma distinction provides a convenient way to circumvent the listing of tissue types when discussing an organ.

- Examples:
  - The parenchyma of the kidney is epithelial tissue (renal tubules and corpuscles). The blood vessels, nerves, and supporting connective tissue of the kidney comprise the stroma.
  - The parenchyma of the heart is muscle tissue (cardiac muscle cells). The nerves, intrinsic blood vessels, and connective tissue of the heart comprise the stroma.
  - The parenchyma of the brain is nervous tissue (nerve cells and glia). The blood vessels within the brain and the connective tissue associated with these blood vessels are stroma.

Wound Healing

Cell Regeneration Capability

Category	Normal replic. rate	Response to injury	Examples
renewing/ labile	High; via stem cell differentiation	modest ↑	skin, intenstinal mucosa, bone marrow
Expanding/ stable	Low	large ↑	endothelium, fibroblasts, hepatocytes, osteoblasts
Static/ permanent	None	No replication	heart muscle cells, nerve cells

1. Labile
   - Continue to proliferate throughout life
   - Epithelial, lymphoid, bone marrow

2. stable (or expanding)
   - Retain this capacity but do not normally replicate
   - Parencymal cells of the liver, kidney, pancreas, endothelium, fibroblast

3. Permanent (or static)
   - Cannot reproduce themselves after birth
   - Nerve and cardiac muscle cells

PERFECT HEALING

FIBROSIS

Cell Regeneration Capability

Biocompatibility

Implant biocompatibility is assessed largely by intensity & duration of the inflammatory response.

Materials Class	Inflammatory response
Metals	very severe in absence of passive oxides
Oxides	minimal
Processed natural polymers	severe
synthetic polymers	mild, unless particulate morphology; additives can give response

1. Chronic inflammation
2. Bacterial Infection
3. Blood Incompatibility
4. Toxicity
5. Tumorigenesis

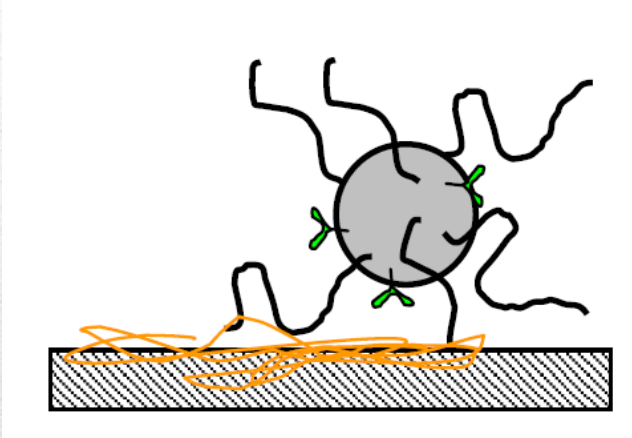
Biomaterial Biocompatibility Concerns

Chronic Inflammation

- Prolonged local chemical or physical irritation—delayed healing
- Often due to **moving parts**, debris, roughness
- Example - PE cup liners in hip replacement implants

Bacterial Infection

- Bacteria compete with cells to adhere to surface
  - similar mechanisms; better adapted to nonviable surfaces
  - resistant to antibiotics (different surface expression)
- Most common bacterial infections:
  - metallic biomaterials: *S. aureus*
  - polymeric biomaterials: *S. epidermidis* (on skin)
  - have receptors for fibronectin & collagen



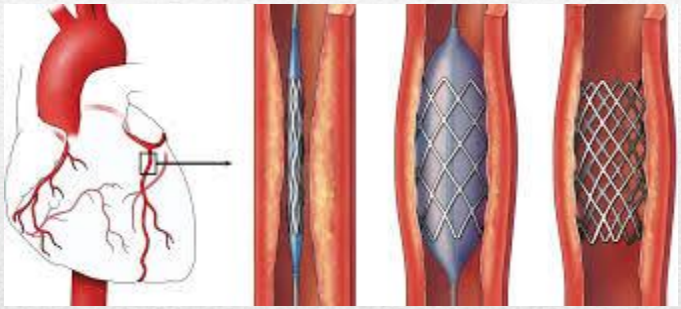
- Example - artificial hearts, synthetic vessels, joint replacement implants, fixation devices, IV catheters, urologic devices, contact lenses
- ~60,000 U.S. deaths/yr from device-related infections urinary catheters, central venous catheters

Blood Incompatibility

- Blood-materials interactions lead to clot or thrombus
  - May compromise device by occlusion

    Example - small (< 5 mm dia.) vascular grafts, stents, IV catheters
  - May detach (embolize) & create vessel occlusion downstream and heart valve removed for copyright reasons.

    Example - emboli to brain from mechanical heart valves ⇒ stroke
  - Susceptible devices require use of anti-coagulation drugs (heparin) ⇒ bleeding risk

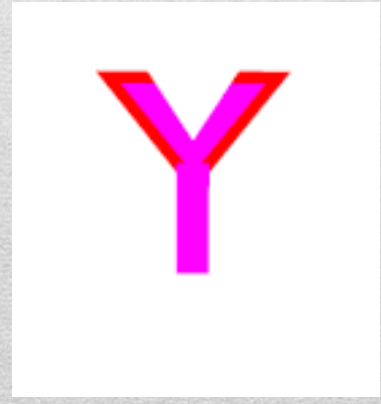


Blood Incompatibility

- Complement activation by extracorpeal therapies
  - C3b adsorption to material ⇒ C5a activation of neutrophils & monocytes (WBCs) to hyperadherent state
  - WBCs stick in lungs ⇒ neutropenia, respiratory distress, hypoxemia (O_2 deficiency—similar symptoms to altitude sickness), tachycardia, cardiac arrest
  - Example - hemodialysis membranes, cardiopulmonary bypass (CPB) devices

Toxicity

- Classical toxicity: from corrosion, degradation or wear products; cytotoxicity increases with amount present
- Immune system toxicity:
  - Immunogenic substances: proteins, carbohydrates, lipids (weakly)
    - Example - processed collagen, natural latex
  - Small molecules (metals, degradation products, drugs) bind on host proteins/cells, making an innocuous substance antigenic
    - Example - hypersensitivity to metals, acrylics



Tumorigenesis

- Rarely observed
- Requires fibrous encapsulation (not seen at chronic inflammation sites)
- Implant role unclear—foreign body reaction may stimulate maturation and proliferation of precancerous cells
- Chemical carcinogens: little supportive data
  - metal implant debris (Cr, Co, Ni) ⇒ carcinogenic in rodents
  - polymer impurities/additives: monomers, solvents, plasticizers, antioxidants

References

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