Lecture 8:
Cell-Surface Interactions: Host Responses to Biomaterials (Part II)

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

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 (Aquired) Immunity

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

Blood proteins:
antibodies (immunoglobulins), cytokines

Cells: lymphocytes (T cells, B cells)

Complement

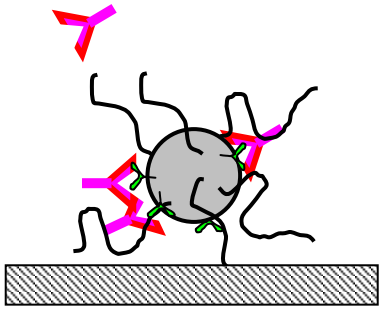
- system of >30 proteins that mediate immune response

- 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)

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

Lectin pathway (discovered in 1990's)**:**

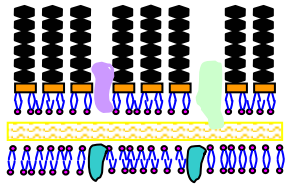
mannan binding lectin (MBL)
binds carbohydrates on pathogen

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)



Alternative pathway:

nonselective pathway of complement (any foreign surface)

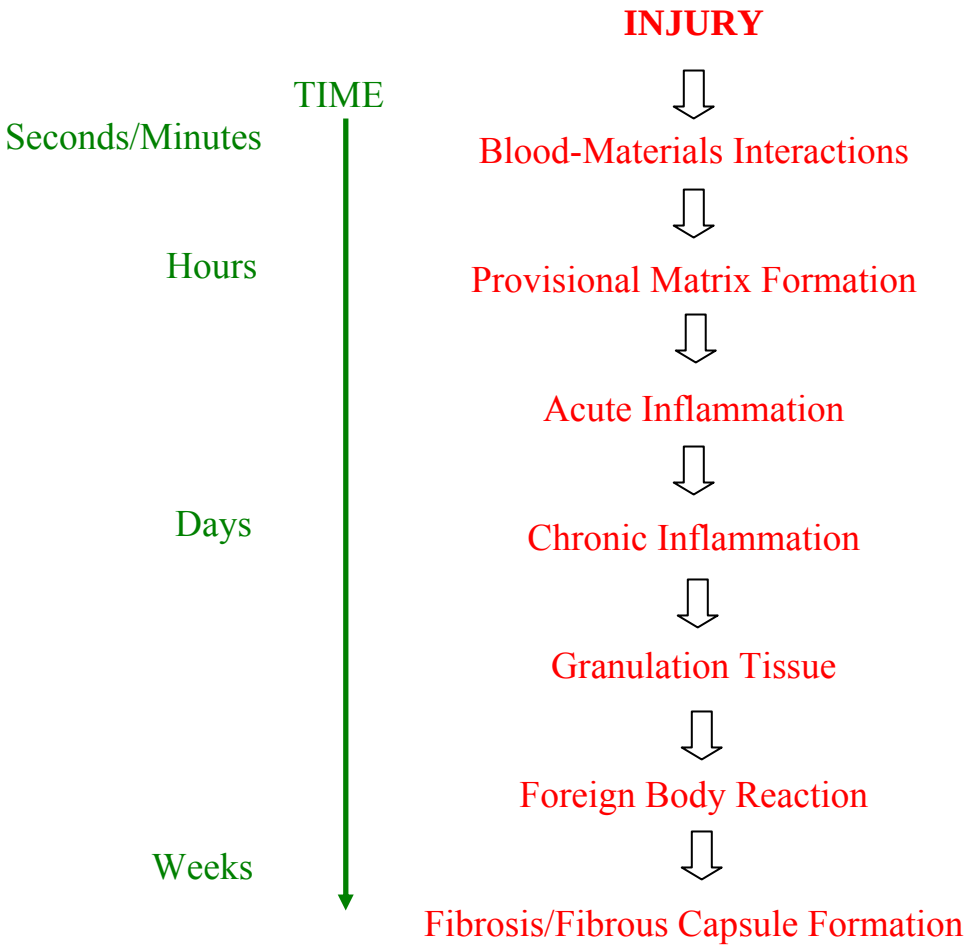
C3 → C3b occurs continuously in plasma at low frequency

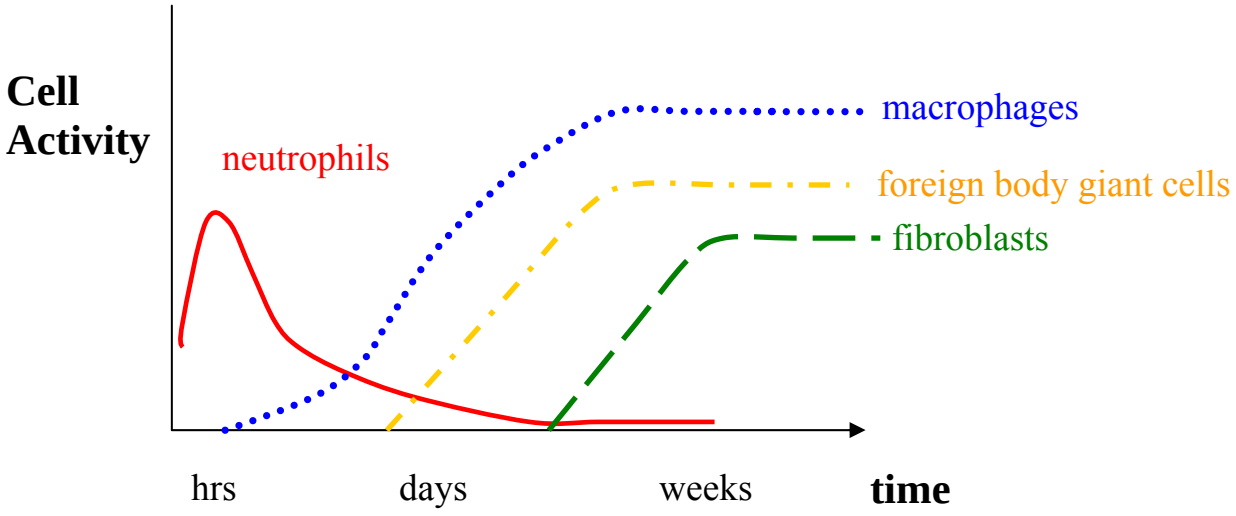
C3b adsorbs on foreign surfaces (biomaterial)

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

Inflammatory Response to Implanted Biomaterials

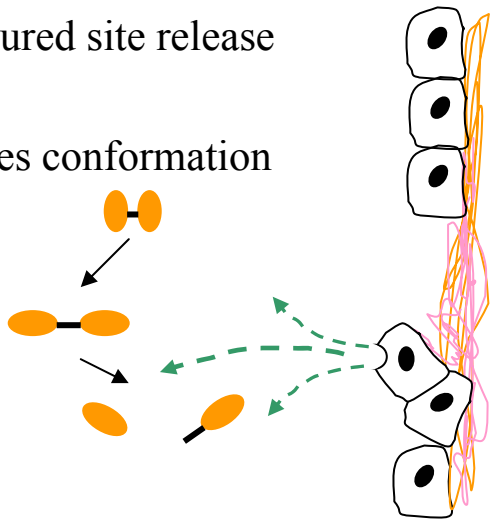




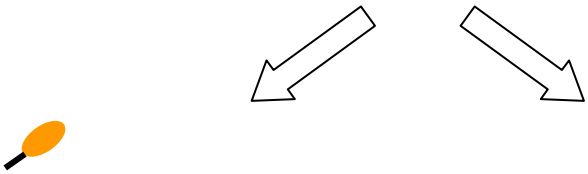
- Endothelial cells lining capillaries near injured site release enzymes

  plasma protein C3 (1.3 mg/ml) changes conformation

  ⇒ cleaved into fragments



C3

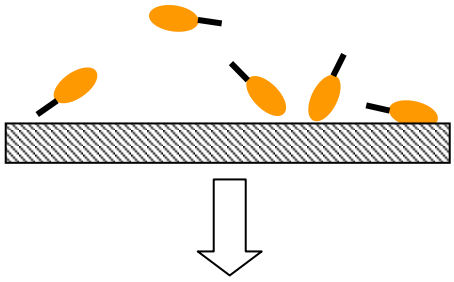


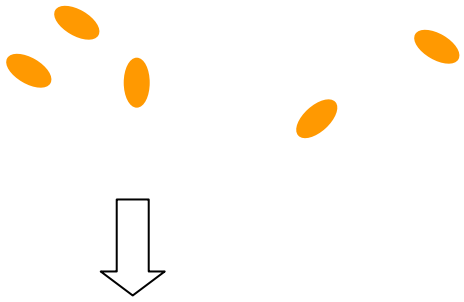
C3b fragment

C3a fragment

C3b attaches to biomtl or injurious agent surface ⇒ insoluble ligand for leukocyte receptors

C3a diffuses into medium ⇒ soluble ligand for leukocyte receptors





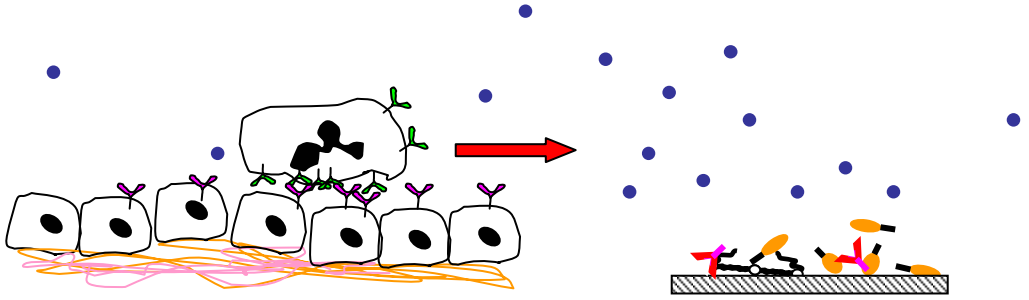
C3b catalyzes C5 cleavage to C5a ⇒ soluble ligand for leukocyte receptors

⇒ **IMMUNE CELL RECRUITMENT**

Neutrophils (PMN's, polymorphonuclear leukocytes)

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

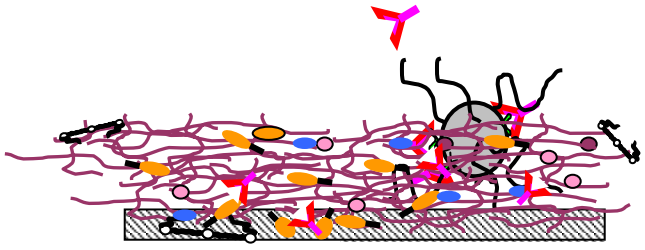
- "first responders" 3-5M/ml (short-lived)
- bind C3a/C5a via complement receptors (CR's)
- become hyperadherent by ↑ CR3 (integrin CD11b/CD18) surface expression – attach to vasculature via endothelial ICAMs
- chemotactic to C5a: migrate to inflammation site



On site, neutrophils bind to C3b, catalyzing release of cytotoxic species: H_2O_2, $O_2^-\bullet$ (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

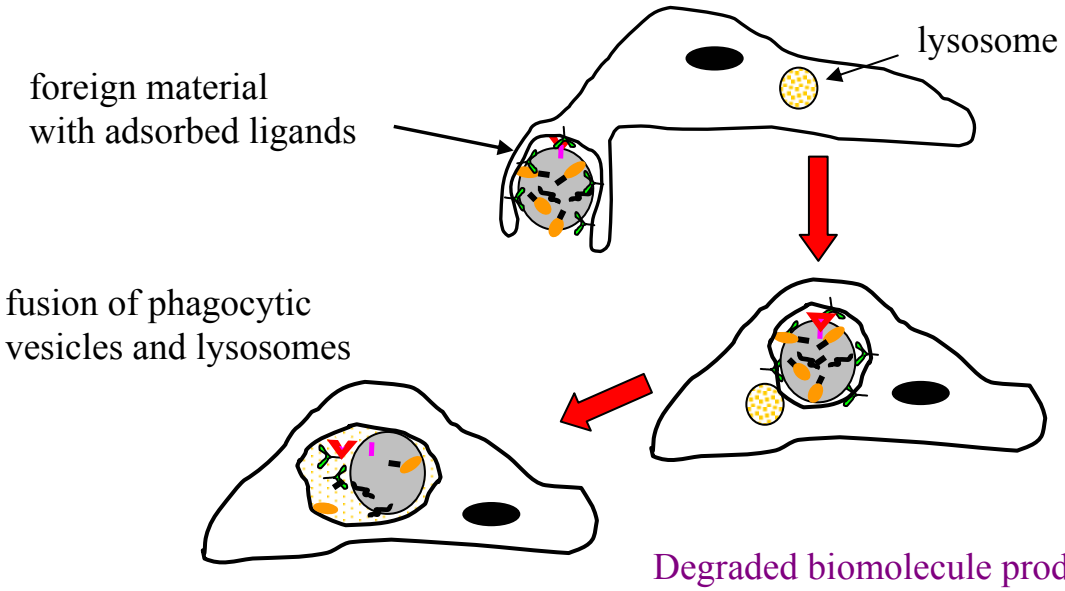
Monocytes (0.2-0.6M/ml)

- 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 response depends on foreign material properties…

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

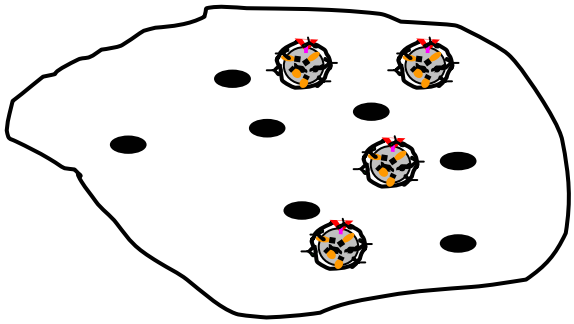


Degraded biomolecule products:
amino acids, sugars, lipids

Nondegradable products accumulate

- 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

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)

Photos removed for copyright reasons.

Fibrous capsule formation around porous P(HEMA-co-MMA) nerve conduit (J.S. Belkas et al., *Biomaterials* **26** (2005) 1741.)

FBGC formed at implant site. Arrows point to nuclei. (J.S. Belkas et al., *Biomaterials* **26** (2005) 1741.)

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

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

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

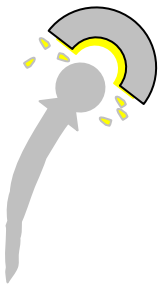
Materials Class	Inflammatory response
Metals	very severe in absence of passive oxides
Oxides	minimal
Processed natural polymers	severe
synthetic polymers	mild, unless particulate morphology; additives can give response

Biomaterial Biocompatibility Concerns

1. Chronic inflammation

- prolonged local chemical or physical irritation—delayed healing
- often due to moving parts, debris, roughness
- proliferation of connective tissue, or tissue necrosis
  (2 extremes of macrophage response)

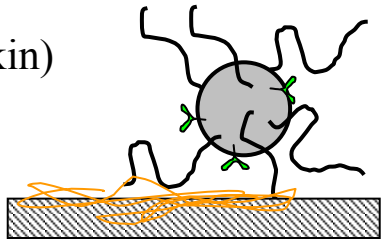
ex. PE cup liners in hip replacement implants



2. 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:
  - polymeric biomaterials: *S. epidermidis* (on skin)
  - metallic biomaterials: *S. aureus*
  - have receptors for fibronectin & collagen



ex. 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

3. Blood Incompatibility

- blood-materials interactions lead to clot or thrombus

  - may compromise device by occlusion
    ex. small (< 5 mm dia.) vascular grafts, stents, IV catheters

Diagrams of stent and heart valve removed for copyright reasons.

  - may detach (embolize) & create vessel occlusion downstream
    ex. emboli to brain from mechanical heart valves ⇒ stroke

  - susceptible devices require use of anti-coagulation drugs (heparin) ⇒ bleeding risk

- 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

ex. hemodialysis membranes, cardiopulmonary bypass (CPB) devices

4. Toxicity

- classical toxicity: from corrosion, degradation or wear products; cytotoxicity increases with amount present

- immune system toxicity:
  i) immunogenic substances: proteins, carbohydrates, lipids (weakly)
  ex. processed collagen, natural latex

  ii) small molecules (metals, degradation products, drugs) bind on host proteins/cells, making an innocuous substance antigenic
  ex. hypersensitivity to metals, acrylics

5. Tumorigenesis

- rarely observed
- morphology dependent vs. chemistry dependent
  (ex. asbestos – needle-like particulates, aspect ratio>100:1)
- requires fibrous encapsulation (not seen at chronic inflammation sites)
- implant role unclear—foreign body reaction may stimulate maturation & 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:

M.A. Horton, Ed., *Molecular Biology of Cell Adhesion Molecules*, John Wiley & Sons: NY, 1996.
D.A. Hammer and M. Tirrell, "Biological Adhesion at Interfaces", *Annu. Rev. Mater. Sci*. 1996, **26**: 651-691.
K.M. Yamada, "Cell Adhesion Molecules", in *Encyclopedia of Molecular Biology*, T.E. Creighton, Ed., John Wiley & Sons: NY, 1999, pp. 361-366.
J.M. Anderson, "Biological Responses to Materials", *Annu. Rev. Mater. Res*. 2001, **31**: 81-110.