The Immune Response

- The reaction to any foreign substance (living or non-living) regardless of pathologic consequences.
- Innate immunity (nonspecific)
- Acquired or adaptive immunity
Innate Immunity

- Physical barriers
- Anti-microbial proteins
- Coagulation factors
- Complement
- Phagocytes (macrophages and neutrophils)
Pathogen-Associated Molecular Patterns (PAMPs)

Pathogens, especially prokaryotes, have molecular structures that

- are not shared with their host;
- are shared by many related pathogens;
- are relatively invariant; that is, do not evolve rapidly
Examples:

- flagellin of bacterial flagella;
- peptidoglycan of gram-positive bacteria;
- lipopolysaccharide (LPS, also called endotoxin) of gram-negative bacteria;
- double-stranded RNA;
- unmethylated DNA.
Pattern Recognition Receptors (PRRs)

- secreted molecules that circulate in blood and lymph;
- surface receptors on phagocytic cells like macrophages that bind the pathogen for engulfment;
- cell-surface receptors that bind the pathogen initiating a signal leading to the release of effector molecules (cytokines).
Complement-Mediated Stimulation of Inflammation

(A) Opsonization and phagocytosis
- Binding of C3b (or C4b) to microbe (opsonization)
- Recognition of bound C3b by phagocyte C3b receptor
- Phagocytosis of microbe

(B) Stimulation of inflammatory reactions
- Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
- Recruitment and activation of leukocytes by C5a, C3a
- Destruction of microbes by leukocytes

(C) Complement-mediated cytolysis
- Binding of C3b to microbe, activation of late components of complement
- Formation of the membrane attack complex (MAC)
- Osmotic lysis of microbe
Complement Activities

- Identification/opsinization of foreign bodies (C3, C4);
- Recruitment/activations (C3a, C5a);
- Lysis of pathogens/cytotoxicity (C5b-9 (MAC);
- Clearing immune complexes and apoptotic cells (C1q, C3b, C4b);
- Augment T and B cell responses (C3, C4, C3a, C5a).
General Features:

AMPLIFICATION: (zymogen cascade)

SOLID-STATE: increases local protein concentration

SOLUBLE SIGNALS: cleaved fragments act as signaling molecules to enhance and regulate inflammation

MULTIPLE INHIBITORS: host cells contain numerous complement inhibitors, inhibitors also present in circulating serum
The Pathways

Figure from Abbas, Cellular and Molecular Immunology, 5th ed 2003
Complement Pathways

Figure from Abbas, Cellular and Molecular Immunology, 5th ed 2003
Late Stages of Complement Activation

- C5 convertase cleaves C5, releases C5a. C5b remains bound
- C5b transiently maintains conformation that binds C6 and C7
- C5bC6C7 is highly hydrophobic, inserts into lipid bilayer
- Binds C8, stabilizes insertion of complex into membrane
- C9 polymerizes at sites of C5b-C8
  - Forms pores (100 angstrom), creates channels
  - Osmotic lysis (rupture)
  - Rapid calcium entry -> activates caspases -> apoptosis
Complement Regulation

- Complement regulatory proteins – soluble and membrane bound
- Importance of rapid regulation of complement – soluble inhibitors abundant in serum
- Cleaved products are normally only reactive for brief periods – ensures limited diffusion and local concentration
  - ex. C3b thioester reactivity is very short-lived

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<td>C4b</td>
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<td>45-70 kD; four CCPRs</td>
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Table 14-7. Regulators of Complement Activation

Abbreviations: CCPR, complement control protein repeat; conc., concentration; GPI, glycoprophosphatidylinositol; MAC, membrane attack complex.
C1 Inhibitor (C1 INH)

• C1 INH is a serine protease inhibitor (serpin class)
• mimics normal substrate of C1r and C1s
• C1q binds antibody, C1r and C1s become active
• C1 INH competes for normal substrate (C4)
• becomes cleaved and attaches to C1 complex
• C1r-C1s tetramer dissociates from C1q
• Limits classical pathway activation

hereditary angioneurotic edema
  • deficiency of C1 INH
  • acute edema in skin and mucosa
  • abdomen pain, vomiting, diarrhea
  • airway obstruction
  • mechanism?
    • over-production of C2 fragment (C2 kinin)
    • remember C1 cleaves C2 when bound to C4b
    • causes excessive vascular permeability
Inhibitors of C3 Convertase

• C3b is commonly deposited on normal host cells
  • remember, C3b is spontaneously generated at low rates
  • if not quickly inhibited, complement will destroy normal host tissue
• Membrane Cofactor Protein (MCP/CD46), Type I Complement Receptor (CR1), Decay Accelerating Factor (DAF), C4-Binding Protein (C4BP)
  • bind to C3b on cell surface
  • competitively inhibit and/or displace binding of other components of the C3 convertase – Bb (alternative path) or C2a (classical path)
  • engineered CR1 used as pharmaceutical
• Factor H is abundant soluble plasma protein (0.5mg/mL)
  • inhibits binding of Bb to C3b
  • Why then does factor H not inhibit C3 convertase formation on microbe surfaces?
    • Factor H has higher affinity for sialic acid rich surfaces
    • Factor H has been applied to biomaterial surfaces

• Paroxysmal nocturnal hemoglobinuria
  • deficiency in enzyme required for forming glycoprophosphatidylinositol-linked membrane proteins (GPI)
  • failure to express DAF, complement-mediated lysis of erythrocytes
  • recurrent intravascular hemolyisis, chronic hemolytic anemia, venous thrombosis
Factor I

- serine protease
- MCP, Factor H, C4BP, and CR1 are cofactors for Factor I cleavage of C3b or C4b
- C3b cleaved fragments generated
  - iC3b, C3d, and C3dg
  - do not activate complement
  - but are recognized by phagocytes

- Thus, further complement activation is halted without affecting leukocyte clearance of foreign particles

- Complement inhibitors can be swamped
  - over-production of complement or antibodies can overcome the inhibitory system
  - results in various disease states
Pattern Recognition Receptors (PRRs)

- secreted molecules that circulate in blood and lymph;
- surface receptors on phagocytic cells like macrophages that bind the pathogen for engulfment;
- cell-surface receptors that bind the pathogen initiating a signal leading to the release of effector molecules (cytokines).
Complement
Complement Activities

- Identification/opsinization of foreign bodies (C3, C4);
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   - Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
   - Recruitment and activation of leukocytes by C5a, C3a
   - Destruction of microbes by leukocytes
General Features:

AMPLIFICATION: (zymogen cascade)

SOLID-STATE: increases local protein concentration as components bind to implant surfaces and promote phagocyte/ macrophage attachment and activation

SURFACE DAMAGE: enzymatic and oxidative reactions.

FRUSTRATED PHAGOCYTOSIS: Macrophages are unable to remove implant.

SOLUBLE SIGNALS: cleaved fragments act as signaling molecules to enhance and sustain inflammation
Mac-1+ Microglia on Retrieved Microelectrodes
ED1 reaction along length of tract
Declining Neurofilament
Neuronal Density and Inflammation

NeuN

GFAP(b) / ED1(g)

200 µm
Neurotoxicity Around Implants?

Questions:

1. Are microglia at the interface of a neural implant chronically activated?

2. Are they neurotoxic?

directed and indirect neuronal cytotoxicity mediated by microglia
Major Activities of Leukocyte Secreted Factors

- Molecules produced in activated macrophages:
  - Phagocyte oxidase
  - iNOS
  - Cytokines (TNF, IL-12)
  - Fibroblast growth factors, angiogenic factors, metalloproteinases
  - Increased MHC molecules, costimulators

- Effector functions of activated macrophages:
  - Killing of microbes
  - Inflammation, enhanced adaptive immunity
  - Tissue remodeling
  - Enhanced antigen presentation
# Inflammation at Biomaterial Interfaces

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>DEVICE</th>
<th>MATERIALS</th>
<th>PHENOMENA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BONE</td>
<td>articulating prosthesis</td>
<td>polyethylene, titanium</td>
<td>osteolysis, loosening of implant</td>
</tr>
<tr>
<td>BLOOD</td>
<td>hemodialysis</td>
<td>cellulose acetate and others</td>
<td>complement deposition, neutropenia,</td>
</tr>
<tr>
<td>SUBCUTANEOUS</td>
<td>breast implants</td>
<td>silicone</td>
<td>fibrosis, calcification, contraction / extrusion</td>
</tr>
<tr>
<td>BRAIN</td>
<td>electrodes</td>
<td>silicon, various metals</td>
<td>encapsulation, loss of chronic recording</td>
</tr>
</tbody>
</table>
## Important Contributing Factors: Plasma Protein Adsorption

**TABLE 1** Properties of the “Big 12” Plasma Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Plasma concentration</th>
<th>Molecular weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/1−mg/ml</td>
<td>μmol</td>
</tr>
<tr>
<td>Albumin</td>
<td>40</td>
<td>600</td>
</tr>
<tr>
<td>IgG</td>
<td>8−17</td>
<td>53−113</td>
</tr>
<tr>
<td>LDL</td>
<td>4.0</td>
<td>2</td>
</tr>
<tr>
<td>HDL</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>α-Macroglobulin</td>
<td>2.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2−3</td>
<td>6−9</td>
</tr>
<tr>
<td>Transferrin</td>
<td>2.3</td>
<td>30</td>
</tr>
<tr>
<td>α-Antitrypsin</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Haptoglobins</td>
<td>2.0</td>
<td>20</td>
</tr>
<tr>
<td>C3</td>
<td>1.6−3.0</td>
<td>8−1.5</td>
</tr>
<tr>
<td>IgA</td>
<td>1−4</td>
<td>7−27</td>
</tr>
<tr>
<td>IgM</td>
<td>0.05−2</td>
<td>0.06−2</td>
</tr>
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*Note:* Numbers used for calculation of \( CD^{1/2} \) are indicated by an asterisk.


Greco, *Implantation Biology*, 1994
Infusion of ovine C5a into sheep mimics the inflammatory response of hemodialysis.

Johnson RJ, Burhop KE, Van Epps DE.

Baxter Healthcare Corporation, Round Lake, IL USA.
Important Contributing Factors: COMPLEMENT

C3 Adsorbed to a Polymer Surface Can Form an Initiating Alternative Pathway Convertase

Jonas Andersson,* Kristina Nilsson Ekdahl,* Rolf Larssen,* Ulf R. Nilsson,* and Bo Nilsson**

In situ complement activation by polyethylene wear debris

David H. DeHeer, 1,2 James A. Engels, 1 Aaron S. DeVries, 1 Robert H. Knapp, 1 John D. Beebe 2
1Grand Rapids Orthopaedic Surgery Residency Program, 1840 Wealthy Street SE, Grand Rapids, Michigan 49506
2Department of Biology, Calvin College, 3201 Burton Street SE, Grand Rapids, Michigan 49546
3Department of Pathology, Spectrum Health East Campus, 1840 Wealthy Street, SE, Grand Rapids, Michigan 49506

• Complement activation by alternative pathway
• Appears independent of “tick-over” pathway
• non-specificic C3 adsorption alone can trigger activation by factor B to generate a functional C3 convertase
• Adsorbed C3 is resistant to factor H and I
• Conformational change upon adsorption is likely cause
• C5a release also detected, potential to initiate leukocyte chemotaxis

2/21/2006

Greco, Implantation Biology, 1994
Important Contributing Factors: FIBRINOGEN

- Fibrinogen deposition on biomaterial surfaces occurs rapidly
- Conformational changes upon adsorption reveal adhesive domains (mimics thrombin mediated conversion to fibrin)
  - Extent varies with material identity
- Mediates macrophage attachment and increased cytokine production
- In this model (PET disc), macrophage attachment was normal in SCID mice (no IgG) and complement depleted mice (cobra venom factor)
- Severe hypofibrinogenemic mice do not mount inflammatory response to PET unless fibrinogen is pre-adsorbed
- Hence, fibrinogen adsorption may be more influential in macrophage attachment than complement or antibodies

Macrophage attachment increases with amount of adhesive epitope exposure in fibrinogen

Mac-1 inhibitor
Important Contributing Factors:
PARTICULATE SIZE AND CONCENTRATION

- Problems in joint prostheses
  - periprosthetic osteolysis
  - chronic inflammation
  - release of wear particles
  - loosening of implant
  - 30,000 revision surgeries/year in U.S.

- debris activates macrophages
  - TNF-alpha release recruits osteoclasts
  - Stimulates NO production -> PGE release
  - Osteoclasts degrade bone
Important Contributing Factors: TNF-alpha

Macrophages consume wear debris and express TNF-alpha surrounding a spinal implant.
Important Contributing Factors: Motion

- prostaglandins are products of cyclooxegenase pathway
- induced by TNF-alpha
- potent pro-inflammatory mediator
- this is still a subject of debate – no definitive in vivo data

Effect of mechanical perturbation on the release of PGE\textsubscript{2} by macrophages \textit{in vitro}


1Department of Orthopaedic Surgery, Brigham and Women’s Hospital, Harvard Medical School, 75 Francis Street, Boston, Massachusetts 02115
2Laboratory for the Study of Skeletal Disorders, Department of Orthopaedic Surgery, Children’s Hospital Medical Center, Harvard Medical School, Boston, Massachusetts 02115

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<td>PGE\textsubscript{2} Release in Response to Mechanical Perturbation (4% Strain) of Nonactivated Macrophages</td>
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<th>Sample</th>
<th>24-h Pre-incubation</th>
<th>24-h Post stretch</th>
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<tr>
<td>Control (n = 3)</td>
<td>119 ± 21</td>
<td>420 ± 122</td>
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<tr>
<td>Stretched (n = 3)</td>
<td>118 ± 26</td>
<td>748 ± 53</td>
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Elastic membrane, 4\% stretch, 1 Hz strain, 1 hour
Figure 1. A schematic representation of the cycle of inflammation around implants from particle-induced osteolysis illustrates macrophage exhaustion, reactive oxygen intermediates, and proinflammatory cytokines recruit a host of local cell types and induce a widening zone of soft tissue damage and inflammation.19
Complement Regulation

- Complement regulatory proteins – soluble and membrane bound
- Importance of rapid regulation of complement – soluble inhibitors abundant in serum
- Cleaved products are normally only reactive for brief periods – ensures limited diffusion and local concentration
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The Wound Healing Continuum

- Initiation by mechanical injury/damage to vasculature
- Blood coagulation-clot formation
- Platelet activation and degranulation
- Inflammation-edema
- Removal of damaged matrix and necrotic cell components
- Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells
- Continued removal of matrix
- Angiogenesis
- Matrix synthesis and deposition
- Epithelialization and wound contraction
- Decrease in cellularity-apoptotic pathway
- Tissue remodeling-elastin synthesis