INFLAMMATION & REPAIR

Lecture 7
Chemical Mediators of Inflammation
Winter 2013

Chelsea Martin

Special thanks to Drs. Hanna and Forzan
i. Inflammation: Introduction and generalities (lecture 1, pp. 1-2)

ii. Classification of inflammation (lectures 2 and 3, pp. 2-8)

iii. Acute Inflammation
i. Vascular events / permeability (lecture 4, pp. 9-11)
ii. Inflammatory cell types (lecture 5, pp. 12-14)
iii. Sequence of cellular events (lecture 6, pp. 15-20)
iv. Chemical mediators (lecture 7, pp. 20-26)

iv. Chronic Inflammation (lecture 8, pp. 27-30)
i. Granulomatous inflammation

v. Repair and wound healing (lecture 9, pp. 31-35)

vi. Healing in specific tissues (lecture 9, cont., pp. 35-37)
CHEMICAL MEDIATORS OF INFLAMMATION

**Definition:** any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response.

**Exogenous**
- endotoxins
CHEMICAL MEDIATORS OF INFLAMMATION

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**Endogenous**
- plasma
- leukocytes
- endothelial cells
- fibroblasts
ENDOGENOUS MEDIATORS

- Preformed mediators in secretory granules
- Newly synthesized
- Factor XII (Hageman factor) activation
- Complement activation

MEDIATORS

- Histamine
- Serotonin
- Lysosomal enzymes
- Prostaglandins
- Leukotrienes
- Platelet-activating factors
- Activated oxygen species
- Nitric oxide
- Cytokines
- Kinin system (bradykinin)
- Coagulation / fibrinolysis system
- \( C_{3a} \)
- \( C_{5a} \)
- \( C_{3b} \)
- \( C_{5b-9} \) (membrane attack complex)
CHEMICAL MEDIATORS - General Principles

• Mechanisms of action
  - receptor-ligand interactions (1\textsuperscript{\circ})
  - direct enzymatic activity
  - oxidative damage

• Network of interacting chemicals
  - high degree of redundancy
  - guarantees amplification & maintenance of inflammatory response

• Short half life

• Potentially harmful
• Vasoactive amines
• Plasma proteases
  – Complement system
  – Kinin system
  – Coagulation / fibrinolytic system
• Arachidonic acid metabolites
• Lysozomal constituents
• Oxygen-derived free radicals
• Platelet activating factor
• Cytokines
• Nitric oxide
Histamine (& serotonin)
- Histamine mainly from mast cells
  - Vasodilation and Increased Vascular Permeability
  - Contraction of non-vascular smooth muscle (bronchi)
  - Stimulate cells to produce eotaxins (attract eosinophils)
What stimulates their release?

- Ag binding to IgE on mast cells
- C3a and C5a
- Direct physical or chemical injury
- Cytokines (IL-1, IL-8)
- Neuropeptides (substance P)
PLASMA PROTEASES

Complement system
- killing system
- vasoactive
- chemotactic

Kinin system
- highly vasoactive
- pain

Clotting / fibrinolytic system
- vasoactive
- cleaves C3

All 3 systems are interrelated
Three pathways:

- Classical pathway (antibodies)
- Alternate pathway (microbe LPS)
- Lectin pathway (sugar on microbes)
Important functions:

- C3a and C5a (anaphylatoxins)
  - release histamine (mast cells) → increased vascular permeability
  - chemotactic for many inflammatory cells

- Opsonize microorganisms to facilitate phagocytosis

- Membrane Attack Complex (MAC) → lysis of pathogens, see movie on website
Bradykinin

- activated by Hageman factor (XIIa)
- generated from plasma proteins (kininogens)
- actions:
  - potent vasodilator
  - stimulates release of histamine (↑ vasc. perm.)
  - contraction of non-vascular smooth muscle
  - produces pain
  - activates the arachidonic acid cascade
Neuropeptides

SUBSTANCE P

Substance P
- secreted by nerve fibers (and some leukocytes)
- burning sensation
- similar functions to bradykinin, related to kinin family
- activated by capsaicins (chiles!)
Factor XII of intrinsic coagulation cascade activated by collagen, etc

- factor XIIa activates:
  - bradykinin
  - coagulation cascade
  - fibrinolytic system
  - complement

- also amplification system
  - kallikrein, plasmin
General conclusions

1. Of all the factors in the multiple systems, the most important are probably…

- Bradykinin, C3a (↑ vascular permeability)
- C5a (↑ permeability and mediator of chemotaxis)
- Thrombin (leads to expression of selectins and adhesion molecules on endothelium, stimulates cytokine production, etc)
General conclusions

2. C3a and C5a generated through several different mechanisms
   - Immunologic pathways involving antibodies and complement (classical pathway)
   - Antibody independent pathways (alternative and lectin pathways)
   - Factors not directly related to immune response (plasmin, kallikrein)
General conclusions

3. Activated Hageman factor (factor XIIa) initiates 4 pathways
   - Kinin system, produces bradykinin
   - Clotting system, produces thrombin (proinflammatory)
   - Fibrinolytic system, produces plasmin and fibrinopeptides (proinflammatory)
   - Complement system, produces anaphylatoxins C3a and C5a
• in both physiologic and pathologic processes (inflammation)

• produced by endothelial cells, leukocytes and platelets

• act locally on smooth muscle, endothelium and platelets

• can mediate most of the steps in acute inflammation!

• origin:
  ➢ arachidonic acid derived from membrane phospholipids (eg linoleic acid)
    ➢ must first be released by activated phospholipases (in injury)

• two important pathways:
  ➢ cyclooxygenase (COX)
  ➢ lipoxygenase
Mechanical, physical, chemical stimuli
Mediators (such as complement)
Cyclooxygenase Inhibitors

- COX-1 is induced by inflammatory stimuli, also constitutively expressed and believed to be responsible for homeostatic functions (renal function, gastric protection)
- COX-2 is induced by inflammatory stimuli

- Non-selective inhibitors (inhibit both COX-1 and -2)
  - Aspirin (irreversible), Ketoprofen (reversible).

- Selective COX-2 inhibitors
  - Meloxicam, Carprofen
  - Expected to be anti-inflammatory without toxicity of nonselective inhibitors
  - May increase risk of arterial thrombosis in people
    - Possibly due to reduced prostacyclin in endothelial cells while sparing of COX-1–mediated production of TxA$_2$ in platelets.
Omega 3 Fatty Acids

• Fish oil is a source of omega 3 fatty acid, and can be consumed in order to modify inflammatory response
• Omega 3 fatty acids serve as poor substrates for cyclooxygenase and lipoxygenase enzymes to synthesize prostaglandins and leukotrienes
• In contrast, omega 3 fatty acids serve as excellent substrates for the production of anti-inflammatory lipid products called resolvins and protectins
Degradation of ECM
  • collagenase, hydrolase, protease (trypsin), elastase

Kill infectious organisms &/or infected cells
  • lactoferrin, lysozyme, myeloperoxidase, major basic protein
  • granzyme/perforin in cytotoxic T lymphocytes
OXYGEN-DERIVED FREE RADICALS

- include: $\text{H}_2\text{O}_2$, $\text{O}_2^-$ and $\text{OH}^-$
- injury to variety of cells (microbes & host cells)
- endothelial damage $\Rightarrow$ increased vascular permeability
- inhibit antiproteases $\Rightarrow$ damage to ECM
PLATELET ACTIVATING FACTOR

- produced by platelets, endothelial cells, leukocytes

- functions:
  - platelet aggregation and release
  - bronchoconstriction & vasoconstriction [high]
  - vasodilation and vascular permeability [low]
  - increases leukocyte adhesion & chemotaxis
  - increases leukocyte degradation / oxidative burst
CYTOKINES

- peptide transmitters for cell-to-cell chatting
  - modulate cell functions
- primarily from activated macrophages & lymphocytes
- esp. IL-1 & TNF-α
IL-1 and TNF - “Master Cytokines”

Microbial products, other cytokines, toxins

ACTIVATION OF MACROSSHAGES
(and other cells)

TNF / IL-1

LOCAL EFFECTS

Vascular endothelium
- ↑ Expression of leukocyte adhesion molecules
- Production of IL-1, chemokines
- ↑ Procoagulant and ↓ anticoagulant activity

Leukocytes
- Activation
- Production of cytokines

Fibroblasts
- Proliferation
- ↑ Collagen synthesis

INFLAMMATION

REPAIR

SYSTEMIC EFFECTS

- Fever
- Leukocytosis
- ↑ Acute-phase proteins
- ↓ Appetite
- ↑ Sleep

SYSTEMIC MANIFESTATIONS OF INFLAMMATION
### Other Cytokines

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<thead>
<tr>
<th>Cytokine</th>
<th>Function</th>
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<tbody>
<tr>
<td>IL-5</td>
<td>eosinophils (chemotaxis, activation, proliferation)</td>
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<tr>
<td>IL-6</td>
<td>B and T cells proliferation (master cytokine)</td>
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<tr>
<td>IL-8</td>
<td>attracts neutrophils (chemokine)</td>
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<td>IF-γ</td>
<td>activates macrophages &amp; T lymphocytes (in viral infections)</td>
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<tr>
<td>PDGF</td>
<td>chemotactic to fibroblasts and leukocytes</td>
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<td>TGF-β &amp; VEGF</td>
<td>important in repair</td>
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NITRIC OXIDE (NO)

- nitric oxide (NO) synthesized from L-arginine by NOS (iNOS in inflammation)
  
- effects: - smooth muscle relaxation → vasodilation
  - reduce platelet aggregation & adhesion
  - bactericidal (forms peroxynitrite)

- uncontrolled NO production (sepsis) → massive vasodilation → shock
CHEMICAL MEDIATORS by EVENT

• Vasodilation
  - histamine, nitric oxide, prostaglandins

• Increased Vascular Permeability
  - histamine, C3a & C5a, bradykinin, ROS, leukotrienes, PAF

• Chemotaxis
  - C5a, LTB₄ & LTC₄ chemokines (TNF-α, IL-1, IL-8), bacterial products (LPS)
CHEMICAL MEDIATORS by EVENT

• Fever
  - IL-1, TNF-α, IL-6, Prostaglandins

• Pain
  - Kinins (Bradykinin, Substance P), Prostaglandins,

• Tissue Damage (leukocyte products)
  - Lysosomal enzymes
  - ROS / ONOO⁻ (NO)
Acute Inflammation Summary

• Resident tissue phagocytes (macrophages) recognize inflammatory inducers such as microbes or dead cells and attempt to eliminate them.
• Phagocytes release cytokines, lipid messengers (arachidonic acid pathway), and other mediators of inflammation.
• Mediators act on vessels to increase permeability and promote edema and recruitment of leukocytes.
• Recruited leukocytes are activated by inflammatory inducers and mediators, and attempt to eliminate the offending agent.
• Elimination of the inflammatory inducer is associated with resolution of inflammation.
• Failure to eliminate the inflammatory inducer results in chronic inflammation.