ACUTE INFLAMMATORY RESPONSE – EXTRAVASATION AND PHAGOCYTOSIS

The accumulation of leukocytes is the most important feature of the inflammatory reaction. Leukocytes engulf and degrade bacteria, immune complexes, and the debris of necrotic cells. Unfortunately, leukocytes may also prolong inflammation and induce tissue damage by releasing enzymes, chemical mediators and toxic oxygen radicals.

SEQUENCE OF LEUKOCYTE EVENTS
- Margination
- Pavementing
- Emigration
- Chemotaxis
- Phagocytosis and Synthesis of biochemical Mediators
- Intracellular Degradation
- Extracellular Release of Leukocyte Products

Notes - Leukocyte Adhesion and Transmigration
- Process regulated by binding of complementary adhesion molecules on the leukocyte and endothelial surfaces. Chemical mediators such as chemoattractants and cytokines affect these processes by modulating surface expression or avidity of such adhesion molecules. Adhesion and migration through vascular endothelium are a prerequisite for cells to get to sites of inflammation. Adhesion molecules are found on the surface of leukocytes and endothelium which bind together to allow leukocytes to adhere to endothelium. There are four main groups of adhesion molecules: Integrin receptor family, Immunoglobulin superfamily, Selectins, and Mucin-like ligands (sialyLe, Lewis)

MARGINATION
- Slowing and stagnation of the flow occurs due to increased vascular permeability
- WBC’s fall out of the central column
- Tumble slowly and roll along the endothelium of venules
- Rest at some point where they adhere
- This is called margination

PAVEMENTING - endothelium appears to be essentially lined by white cells

E-selectin (on endothelial cells), P-selectin (endothelial cells and platelets), and L-selectin (on most leukocytes), bind through their lectin domain to sialylated forms of oligosaccharides which are bound to various mucin-like glycoproteins.

Immunoglobulin superfamily includes two endothelial adhesion molecules (ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1). These molecules serve as ligands for intregrins (which are found on leukocytes)
Integrins – transmembrane heterodimeric glycoproteins with \( \beta \) and \( \alpha \) subunits. Expressed on many cell types and bind to other cells including endothelial cells and the extracellular matrix. The \( \beta_2 \) integrins (LFA-1 and MAC-1) bind to ICAM-1. The \( \beta_1 \) integrins (VLA-4) bind to VCAM-1. These molecules are expressed on the surface in a low-affinity state. (Many more integrins are known – but these are the two major ones we will worry about.)

Mucin-like glycoproteins (M-LG)– These molecules serve as ligands for the leukocyte adhesion molecule CD44. M-LG are found on other cell surfaces and within the ECM. Heparin sulfate is one example.

- Chemical mediators (histamine, thrombin, platelet activating factor (PAF) stimulate the redistribution of P-selectin from intracellular granules to the cell surface. This occurs early in the inflammatory process.
- Other chemical mediators (TNF and IL-1 and chemokines) are secreted by resident macrophages, mast cells and endothelial cells as a response to injury. These induce the post-capillary venule to express adhesion molecules. Within 1-2 hours endothelial cells express E-selectin.
- Selectins bind to lectins on the microvillar tips of leukocytes – but this is a low-affinity interaction and can easily be detached. This results in rolling.
- TNF and IL-1
  - Induce endothelial expression of ICAM and VCAM
  - Bind to proteoglycans on endothelial cells and activate leukocytes
- Activated leukocytes
  - VLA-4 and LFA-1 go from low to high affinity state
  - Bind to ICAM-1 and VCAM-1 on endothelial surface
Leukocytes stop rolling and spread out over surface of endothelial cell

**Endothelial/Leukocyte Adhesion Molecules**

<table>
<thead>
<tr>
<th>Endothelial Molecule</th>
<th>Leukocyte Receptor</th>
<th>Major Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin</td>
<td>Sialyl-Lewis X</td>
<td>Rolling (neutrophils, monocytes, lymphocytes)</td>
</tr>
<tr>
<td></td>
<td>PSGL-1</td>
<td>PSGL-1 = P-Selectin Glycoprotein Ligand 1</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Sialyl-Lewis X</td>
<td>Rolling, adhesion to activated endothelium (neutrophils, monocytes, T cells)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>CD11/CD18 (integrins)</td>
<td>ICAM-1 = Intercellular Adhesion Molecule-1</td>
</tr>
<tr>
<td></td>
<td>(LFA-1, Mac-1)</td>
<td>Adhesion, arrest, transmigration (all leukocytes)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>( \cdot 4 \cdot 1 ) (VLA4) (integrins)</td>
<td>VCAM-1 = Vascular Cell Adhesion Molecule-1 (eosinophils, monocytes, lymphocytes)</td>
</tr>
<tr>
<td></td>
<td>( \cdot 4 \cdot 7 ) (LPAM-1)</td>
<td></td>
</tr>
<tr>
<td>GlyCam-1</td>
<td>L-selectin</td>
<td>Lymphocyte homing to high endothelial venules</td>
</tr>
<tr>
<td>CD31 (PECAM)</td>
<td>CD31</td>
<td>PECAM = Platelet Endothelial Cell Adhesion Molecule (immunoglobulin superfamily) Leukocyte migration through endothelium</td>
</tr>
</tbody>
</table>

*ICAM-1, VCAM-1, and CD31 belong to the immunoglobulin family of proteins
Modified from: Robbins and Cotran Pathologic Basis of Disease, 7th ed, Kumar, Abbas & Fausto, p. 54, 2005.*
EMIGRATION

Definition: Process by which leukocytes escape from their location in the blood to reach the perivascular tissues, (sometimes referred to as Diapedesis...)

Process:
1. After adhesion - leukocytes move along the endothelial surface
2. Insert large cytoplasmic extension pseudopodia into endothelial gaps
3. Gaps created by actions of histamine and other chemical mediators as well as by the leukocytes themselves
4. PECAM – adhesion molecule is important in this process
5. Entire cell passes through once pseudopodia are through
6. Collagenase breakdown basement membrane (rarely >1: )

Location: Occurs in the postcapillary venule because:
- Adequate number of inter-endothelial gaps
- Adequate number of receptors - particularly histamine

Time:
Mild Inflammation
- Neutrophils peak 4-6 hours
- Mononuclear cells peak 18-24 hours
- Emigration begins when PMN’s \ and lasts longer
CHEMOTAXIS

Definitions:
- **Chemotaxis**: Directional migration in response to a chemical gradient of chemoattractant. The process is receptor-mediated. Chemotaxis implies directed locomotion.
- **Chemokinesis**: Enhanced random movement.
- **Chemotaxins and Chemokines**: Mediators which make leukocytes travel chemoattractants.

CHEMOATTRACTANTS FOR LEUKOCYTES

Plasma-derived
- C5a, C5a des-Arg
- Fibrin degradation products

Inflammatory Cell derived
- LTB$_4$, HETE’s - from arachidonic acid metabolism
- PAF, cytokines – IL-8

Others
- Bacterial - fMLP-like peptides, lipid products
- Dead cells – necrotaxis

Chemokines - important for selectivity of the inflammatory reaction
- Small proteins divided into families. A form of cytokine.

<table>
<thead>
<tr>
<th>Family</th>
<th>Examples</th>
<th>WBC attracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;-chemokines group A (with ELR)</td>
<td>IL-8</td>
<td>Neutrophils - attracted and activated</td>
</tr>
<tr>
<td>&quot;-chemokines group A (without ELR)</td>
<td>PF4</td>
<td>Leukocytes but not neutrophils</td>
</tr>
<tr>
<td>$-$chemokines</td>
<td>RANTES, Eotaxin</td>
<td>Leukocytes but not neutrophils</td>
</tr>
<tr>
<td>“C” Chemokines</td>
<td>Lymphotactin</td>
<td>“Resting” T lymphocytes</td>
</tr>
<tr>
<td>C-XXX-V Chemokine</td>
<td>Fractalkine</td>
<td>NK cells</td>
</tr>
</tbody>
</table>

Mechanisms of Chemotaxis

- Leukocytes crawl - require adhesive surface
- Undergo morphological shape changes
- Release of calcium
- Migrate towards the highest concentration of chemoattractant (Adherence, secretion and locomotion)

Microtubules - allows cell to orient toward the chemotactic gradient
Microfilaments - responsible for the movement
Leukocyte Activation Mechanisms and Functions

Leukocyte Activation Responses

- Production of arachidonic acid metabolites from phospholipids by activation of phospholipase A
- Degranulation and secretion of lysosomal enzymes
- Activation of oxidative burst
- Secretion of cytokines to amplify and regulate the inflammatory process
- Modulation of leukocyte adhesion molecules
- Chemotaxis
- Elaboration of arachidonic acid metabolites
- Secretion

PHAGOCYTOSIS AND INTRACELLULAR DEGRADATION

**Purpose** - engulf, kill and degrade foreign material (usually bacteria)

Phagocytosis consists of:

1. **Recognition and attachment** of bacteria
   a. Important Receptors
      i. Mannose Receptor
      ii. Scavenger Receptor
   b. Opsonized bacteria Specific Receptors
      i. Antibody (Fc fragment)
      ii. Fibronectin
      iii. C3b

2. **Engulfment**:
   - Small cytoplasmic extensions or pseudopods extended by cell
   - Flow around the attached particle until it is engulfed
   - Cytoplasmic processes pinch together, meet, and fuse
   - Form a **phagosome**

3. **Phagolysosome formation**
   - Fusion of lysosomal granules with phagosome
   - Degranulation of lysosomes into phagosome
   - Cellular mechanisms similar to that of chemotaxis

*"The phagocytes won't eat the microbes unless the microbes are nicely buttered for them" (George Bernard Shaw: The Doctor's Dilemma)."
Intracellular killing and degradation

Two categories of bactericidal mechanisms are recognized
1) Oxygen-dependent mechanisms
2) Oxygen-independent mechanisms

Oxygen-dependent mechanisms - production of reactive oxygen intermediate species

Respiratory burst of phagocytosis
1) 2-3 \( \text{O}_2 \) consumption
2) \( \text{O}_2^- \) superoxide anion generation
3) \( \text{H}_2\text{O}_2 \) production
4) \( \text{O}_2 \) glucose metabolism via hexose monophosphate shunt (HMPS)

Important enzymes responsible for respiratory burst

NADPH oxidase
Present in lysosomal membrane - now fused with phagosome

\[
2 \text{O}_2 + \text{NADPH} \rightarrow 2 \text{O}_2^- + \text{NADP}^+ + \text{H}^+
\]
Products:
1. NADP required for HMPS (produces more NADPH)
2. \( \text{O}_2^- \) spontaneous becomes \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \)

These oxygen metabolites are the principal killers of bacteria

1. **The \( \text{H}_2\text{O}_2 \)-Myeloperoxidase-Halide System (Myeloperoxidase-dependent killing)**
   
   The quantities of \( \text{H}_2\text{O}_2 \) produced by the phagolysosomes are insufficient to induce effective killing of bacteria. The azurophilic granules of neutrophils contain the enzyme myeloperoxidase (MPO), which in the presence of a halide such as \( \text{Cl}^- \), (or iodine or bromide) converts hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) to hypochlorite (\( \text{HOCl}^- \)), a powerful oxidant and antimicrobial agent. A similar mechanism is also effective against fungi, viruses, protozoa, and helminths.

   \[
   \text{H}_2\text{O}_2 + \text{Cl}^- + \text{MPO} \rightarrow \text{HOCl}^- + \text{H}_2\text{O} + \text{Cl}^-
   \]

2. **Haber-Weiss Reaction**

   \[\text{O}_2^- + \text{H}_2\text{O}_2 + 6\text{Fe}^3+ + 6\text{H}^+ \rightarrow 6\text{OH}^- + \text{OH}^+ + \text{O}_2\]

   Requires iron
   
   hydroxyl ions (\( \text{OH}^+ \)) - extremely reactive oxidant

3. **Nitric Oxide** and production of peroxynitrite via superoxide anion

```
  2 \text{O}_2 + \text{NADPH} \rightarrow 2 \text{O}_2^- + \text{NADPH} + \text{H}^+

  \text{NADPH} + \text{Fe}^{3+} \rightarrow \text{HOCl}^- + \text{H}_2\text{O}_2

  \text{SUMMARY OF OXYGEN-DEPENDENT BACTERIAL MECHANISMS}
```
Oxygen Independent Mechanisms - Substances within leukocyte granules

**Lysozyme** - attacks bacterial cell walls - especially gram + bacteria (specifically hydrolyzes muramic acid-N-acetyl-clucosamine bond present in the glycopeptide coat of all bacteria.

**Bactericidal permeability increasing protein (BPI)**, a highly cationic granule-associated protein causes phospholipase activation, phospholipids degradation and increased permeability of the outer membrane of microorganisms.

**Lactoferrin** - iron binding glycoprotein prevents use of iron by bacteria

**Defensins** (cationic arginine-rich granule peptides)
- Cytotoxic to microbes and certain mammalian cells

**N**<sup>ramp1</sup> protein (Natural resistance-associated macrophage protein one)
- Transporter function
- 8 cytoplasmic flux of iron (helps phagolysosome starve bacteria of iron)

**Major Basic Protein**
- Cationic protein of eosinophils
- Limited bactericidal activity
- Cytotoxic to many parasites

**Cathepsin G** - protease within azurophilic granules
- Antimicrobial properties for both Gram-positive and Gram-negative bacteria and some fungi

Lysosomal enzymes

**Degradation:**
- pH of the phagolysosome drops (pH 4-5) after phagocytosis
- This is the optimal pH for the action of degradative enzymes within lysosomes.

Survival of Phagocytosed Microorganisms

Almost all microorganisms are killed following phagocytosis - unless host affected with a defect in phagocytosis or killing

- eg: **Chronic granulomatous disease of childhood**: Children with this have neutrophils incapable of producing \( \text{H}_2\text{O}_2 \) during phagocytosis and results in recurrent infections.

Some organisms survive within phagolysosome
- eg: **Mycobacterial sp., Coxiella burnette**

Some escape phagolysosome and grow in cytoplasm
- eg: **Rikettsiae, Listeria monocytogenes**

Some don’t allow lysosome to fuse with phagosome
- eg: **Toxoplasma gondii, M. tuberculosis, Salmonella enterica, Legionella pneumophila**

Tissue Damage Resulting During Phagocytosis

3 basic mechanisms whereby phagocytic cells release their potent chemical and release tissue at sites of inflammation and thereby contribute to inflammation:

**Important Tissue Toxins**
1. Lysosomal enzymes
2. Oxygen-derived active metabolites
3. Products of arachidonic acid metabolism
   (prostaglandins and leukotrienes)

**Lysosomal Suicide** - **Cytotoxic release** (2 ways this can occur)
1. Pathogenic bacteria overwhelms the leukocyte •
   Phagolysosome may rupture into the cytoplasm •
   Releasing spilling out potent hydrolytic lysosomal enzymes •
   Kill cell
2. Leukocyte dies •
   Releases lysosomal enzymes into local environment •
   Producing exudate see in inflammatory conditions

Regurgitation during feeding
   - Fusion of lysosome occurs before phagosome is closed
   - Seen in “feeding frenzies”
   - Lysosomal contents released to environment
   - Producing enzymatic damage to host tissues

Reverse endocytosis (frustrated phagocytosis)
   Phagocytic stimuli too large to be internalized
eg: bacteria on a fibrin meshwork
eg: complexes of antigen antibody fixed against a basement membrane
eg: immune complexes on joint surface
Can not form a phagosome
Discharge of lysosomal contents (In frustration!)

Termination of the acute inflammatory response
- Mediators have short half lives
- Mediators are degraded after release
- Produced in short bursts when stimulus persists
- Switch to anti-inflammatory lipoxins from arachidonic acid
- Production of anti-inflammatory cytokines (TGF-β)
- Inhibit the production of TNF in macrophages

CHEMICAL MEDIATORS OF INFLAMMATION

General Principles:
- Originate from plasma of cells. When in plasma are found in inactive state and must be activated. When in cells are often within granules and need to be secreted or are synthesized in response to a stimulus.
- Production of active mediators is triggered by microbial products or host proteins.
- Most require binding to specific receptors on target cells for biologic activity.
- Some have direct enzymatic activity,
- One mediator can stimulate the release of other mediators by target cells, (provide mechanism of amplification).
- May have different affects on different cells.
- Most are short lived.
- Many have the potential to be harmful.

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

The mediation of inflammation comprises an extensive network of interacting chemicals that render the system with a high degree of redundancy. This guarantees that both amplification and preservation of the response can be maintained even if one component or another of the system is deficient. There is almost no part of the inflammatory response that is dependent on a single mediator.
Most Common Mediators in Inflammation

<table>
<thead>
<tr>
<th>Category</th>
<th>Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasodilation</td>
<td>Prostaglandins, Nitric Oxide</td>
</tr>
<tr>
<td>Increased Vascular Permeability</td>
<td>Vasactive amines, C3a and C5a, Bradykinin, Leukotrienes C4, D4, E4, Platelet-activating factor</td>
</tr>
<tr>
<td>Chemotaxic Leukocyte Activation</td>
<td>C5a, Leukotriene B4, Chemokines (eg IL-8)</td>
</tr>
<tr>
<td>Fever</td>
<td>IL-1, IL-6, TNF-α, Prostaglandins</td>
</tr>
<tr>
<td>Pain</td>
<td>Prostaglandins, Bradykinin</td>
</tr>
<tr>
<td>Tissue Damage</td>
<td>Neutrophil and macrophage products, lysosomal enzymes, Oxygen metabolites, Nitric oxide</td>
</tr>
</tbody>
</table>

1. **Vasoactive amines**: histamines and serotonin
2. **Plasma proteases**:
   1. The complement system (C3a; C5a; C5b-C9)
   2. The kinin system (bradykinin and kallikrein)
   3. The coagulation-fibrinolytic system (fibrinopeptides, fibrin degradation products)
3. **Arachidonic acid (AA) metabolites**:
   1. Via cyclooxygenase (endoperoxides, prostaglandins, thromboxane)
   2. Via lipoxygenase (leukotriene; HPETE; HETE)
4. Lysosomal constituents (proteases)
5. Oxygen-derived free radicals
6. Platelet activating factor (PAF)
7. Cytokines
8. Nitric oxide
9. Growth factors

**Vasoactive Amines**
Histamine and serotonin are believed to be the primary mediators in the immediate active phase of increased permeability. Vasoactive amines cause vasodilation and increased vascular permeability by causing endothelial cells to round up and increase vesiculovacular transfer of fluids. These chemicals are stored within cells for immediate release.

**Histamine** is extensively distributed in tissues, the main source being the mast cells that are normally present in the perivascular connective tissue. It is also present in
granules of basophils and in platelets (some species). Histamine is important mainly in early inflammatory responses and in immediate Ig-E mediated hypersensitivity reactions. It is preformed and stored in granules with heparin. Histamine is important in the immediate active phase of increased vascular permeability, promotes contraction of extravascular smooth muscles in the bronchi and stimulates stromal cells to synthesize and release eotaxins, (chemotaxins for eosinophils).

The following agents can stimulate release of histamine from mast cells:
- Physical injury, mechanical trauma, heat, chemical agents
- Snake venoms, toxins, bile salts, ATP
- Immune reactions involving binding of antibodies to mast cells
- Fragments of complement called anaphylatoxins (C3a and C5a)
- Histamine-releasing factors from neutrophils, monocytes, and platelets
- Cytokines (interleukin-1, IL-8)
- Neuropeptides

**Serotonin** is a vasoactive mediator, present in platelets and some mast cells (not in humans). Serotonin has the following characteristics:
- Acts primarily on venules
- Important in early phase of acute inflammation
- It is released from mast cells, basophils and platelets

The release of histamine and serotonin from platelets (the platelet release reaction) is stimulated when platelets aggregate after contact with collagen, thrombin, ADP, and antigen-antibody complexes.

**PLASMA PROTEINS** – Three various and interrelated systems important in the inflammatory response are found within plasma. These systems include the complement, kinin and clotting systems.

**Complement system**: Consists of activating and effector sequences consisting of 20 protein components most common in plasma. The system functions both in innate and adaptive immunity. This plasma protein acts primarily as a defense mechanism against microbial agents. The products of complement activation are involved in:
- Vascular permeability (C3a, C5a and C4a (lesser extent) – cause histamine release from mast cells. Referred to as anaphylatoxins.
- Chemotaxis – C5a chemoattractant for neutrophils, monocytes, eosinopils and basophils.
- Opsonization prior to phagocytosis
- Lysis of target organisms

C3 and C5 are the most important inflammatory mediators. In addition to activation of the complement system, several proteolytic enzymes in the inflammatory exudates (plasmin, lysosomal enzymes), can also activate these proteins.
The Kinin System: The kinin system is one of three mediator systems triggered by contact activation of Hageman factor. The kinin system generates vasoactive peptides from plasma proteins called kininogens by the action of specific proteases called kallikreins. This system results in the ultimate release of the vasoactive nonapeptide bradykinin. Bradykinin has the following actions:

* Potent vasodilator
* Increased vascular permeability
* Contraction of smooth muscle
* Produce pain
* Stimulate release of histamine from mast cells
* Activate the arachidonic acid cascade

Clotting system: We have previously discussed coagulation in Hemostasis. However the clotting system and inflammation are intimately connected. The intrinsic clotting system is a sequence of plasma proteins that can be activated by Hageman factor (factor X11 – produced in liver and circulating in inactive form). The final phase of the cascade is the conversion of fibrinogen to fibrin by the action of thrombin. Fibrin binds to protease-activated receptor 1 (PAR-1) a G protein-coupled receptor on platelets, endothelial cells and smooth muscle cells as well as other cells. This binding produces the following:

1. Mobilization of P-selectin
2. Production of chemokines
3. Expression of endothelial adhesion molecules for WBC integrins
4. Induction of cyclooxygenase-2 – production of prostaglandins
5. Production of platelet activating factor
6. Production of nitric oxide
7. Changes in endothelial shape

Factor XIIa also activates the fibrinolytic system. Plasmin can activate C3 (coagulation) 
Fibrinopeptides are formed, which may induce increased vascular permeability and be chemotactic activity for leukocytes.

**NOTE:** Activated Hageman factor (factor XIIa) initiates the clotting, fibrinolytic and kinin systems. The products of this initiation (kallikrein, factor XIIa, and plasmin, but particularly, kallikrein) can, by feedback, activate Hageman factor, resulting in significant amplification of the effects of the initial stimulus.

![Diagram of the fibrinolytic system](image)

**Figure 2-15**

Interrelationships between the four plasma mediator systems triggered by activation of factor XII (Hageman factor). Note that thrombin induces inflammation by binding to protease-activated receptors (principally PAR-1) on platelets, endothelium, smooth muscle cells, and other cells. *from: Robbins and Cotran Pathologic Basis of Disease, 7th ed, 2004 p 67.*

**ARACHIDONIC ACID METABOLITES**

When cells are activated by diverse stimuli, their lipid membranes can be rapidly remodeled to generate biologically active lipid mediators which have a variety of biologic processes some of which are seen in inflammation and Hemostasis. These lipid mediators are thought of as short-range hormones that are formed rapidly and exert their effects locally and then are inactivated.

Oxygenated arachidonic acid derivatives have roles in a variety of biologic and pathologic processes, only one of which is inflammation. Arachidonic acid is a 20-carbon polyunsaturated fatty acid that is derived directly from dietary sources or by conversion from the essential fatty acids like linoleic acid. It does not occur free in the cell but is normally esterified in...
membrane phospholipids. In order for arachidonic acid to be utilized by the cell, it must first be released from phospholipids. This is accomplished through the activation of cellular phospholipases, particularly phospholipase A₂, via mechanical, chemical and physical stimuli or by other mediators. Following activation, biosynthesis of the metabolites of arachidonic acid occurs by one of two major classes of enzymes: pathways: the **cyclooxygenase pathway** and the **lipoxygenase pathway**. These arachidonic acid metabolites are collectively termed eicosanoids.

**Cyclooxygenase Pathway** – produce prostaglandins

2 Enzymes are able to produce these products:

- **COX-1 and COX-2**
- COX-1 normally present and necessary for everyday activities and is synthesized at sites of inflammation
- COX-2 is transcriptionally regulated - present in various circumstances such as inflammation

3 products are important:

- C Thromboxane A₂ found in platelets and other cells is a potent platelet aggregator and vasoconstrictor
- C Prostacyclin (PGI₂), found predominantly in endothelial cells, is a potent inhibitor of platelet aggregation and vasodilator.
- C Prostaglandins PGE₂, PGF₂α and PGD₂, which have a variety of actions on vascular tone and permeability and can cause pain

**NOTE**: Drugs such as corticosteroids, aspirin and indomethacin have anti-inflammatory properties because they inhibit the cyclooxygenase pathway of arachidonic acid (inhibit biosynthesis of prostaglandins).

**Lipoxygenase Pathway** – enzymes not found in all cells.

The most important metabolites are **leukotrienes**, so designated because of their conjugated triene chain and their initial isolation from leukocytes.

- **Leukotrienes**: Leukotriene B₄ is a potent chemotactic agent. Leukotrienes C₄, D₄, E₄ are very potent vasoconstrictors, they also act as potent mediators of increased vascular permeability on venules only. These leukotrienes are up to 1000 times as potent as histamine in producing increased vascular permeability. Old name for these compounds was “slow reacting substances of anaphylaxis.” Also cause bronchospasm.

**Lipoxins** – Leukocytes, primarily neutrophils, produce intermediates in lipoxin synthesis which are converted to lipoxins by platelets interacting with leukocytes. Lipoxins A₄ and B₄ are generated. The principle action is to inhibit leukocyte recruitment and cellular components of inflammation. Inhibit neutrophil chemotaxis and adhesion to endothelium.
PLATELET ACTIVATING FACTOR (PAF)

- Bioactive phospholipids.
- Produced by a variety of cells, including platelets, basophils, mast cells, neutrophils, monocytes, macrophages and endothelial cells following stimuli.
- Produces effect via a single G-protein-coupled receptor:
  * Platelet aggregation and release
  * Bronchoconstriction and vasoconstriction
  * Vasodilation and increased vascular permeability (100-10,000 X histamine)
  * Increased leukocyte adhesion to endothelium
  * Leukocyte chemotaxis, degranulation and oxidative burst

CYTOKINES and CHEMOKINES

Cytokines are polypeptide factors produced by many cells (but principally activated macrophages and lymphocytes) that modulate the function of other cell types. Cytokines are essential transmitters of cell-to-cell communication in many physiological and pathophysiological processes. Cytokines that appear to be important mediators of inflammation are Interleukin-1 (IL-1), tumor necrosis factor (TNF), Interleukin-6 (IL-6) and Interleukin-8 (IL-8), Interferon (IFN-α), chemokines, colony stimulating factors (CSF). (See Table 4-16 page 208, Slauson and Cooper)

IL-1 and TNF are monocyte-macrophage derived cytokines, they are biochemically and immunologically distinct proteins, but are similar in their biologic activities. IL-1 and TNF have the following inflammatory effects: “Master Cytokines”

C On endothelial cells, these cytokines increase leukocyte adhesion (induction of surface antigens), stimulate the synthesis of PGI₂ and PAF, and increase procoagulant activity (surface thrombogenicity).
They induce systemic **acute phase responses**, including fever, neutrophilia, hemodynamic effects (shock), acute phase proteins and slow-wave sleep.

**On fibroblasts**, they induce proliferation, increased collagen formation, and increased collagenase and protease synthesis.

**IL-5** has an effect on proliferation, chemotaxis, and activation of eosinophils, resulting in eosinophilic in parasitic infections, hypereosinophilic syndrome, etc.

**IL-6.** The major biological activities of IL-6 include: stimulated both B and T cell proliferation. “Master cytokine” similar to IL-1 and TNF.

**IL-8** is a powerful chemoattractant and activator of neutrophils and to lesser degree monocytes and eosinophils- so really is a chemokine.

**IFN-** – activates macrophages

Cytokines that simulate hematopoiesis – Colony Stimulating Factors (CSF)

**GROWTH FACTORS:** Platelet-derived growth factor (PDGF) and transforming growth factor-beta may be chemotactic to leukocytes and mesenchymal cells and have activities resembling those of cytokines. Growth factors are particularly important in tissue regeneration and repair.

**NITRIC OXIDE (NO):** Tiny uncharged molecule. Produced in many cell types and has many functions. Best-known effects (3)

1) Ð - potent physiological mediator of vascular tone - particularly vasodilation
2) Effector of host defense against certain pathogens
3) Signalling molecule - especially in brain
4) Reduces platelet aggregation and adhesion
5) inhibits several features of mast cell-induced inflammation

-Cells need the enzyme nitric oxide synthase (NOS) and NADPH, FAD, FMN, BH₄ to form NO. Like COX-1 and COX 2 there are isoforms, which are either continuously produced (iNOS) or inducible (cNOS). When the inducible NOS is upregulated within endothelial cells (eg. Septicemia) may see massive vasodilation. In macrophages NOS is induced when macrophages are activated by cytokines.

**Some actions with regard to inflammation**

- Formation of free radical: NO + O₂ − 6666 ONOO⁻ (peroxynitrite)
- Inhibitory and/or destructive to many bacteria

**Hageman Factor - Dependent Pathways**

(Factor XII of intrinsic coagulation cascade)

- Activation by:
  - Contact with collagen
  - Activated platelets
  - Proteases from neutrophils and other leukocytes

- Results in:
  - Blood clotting
  - Fibrinolytic system activated
  - Production of kinins
  - Activation of complement cascade
  - Feedback system
LYSOSOMAL CONSTITUENTS.
Neutrophils exhibit two major types of granules:
C Specific - contain lactoferrin, lysozyme, alkaline phosphatase and collagenase
C azurophilic - contain myeloperoxidase, cationic proteins, acid hydrolases, and neutral proteases
Monocyte granules contain acid hydrolases, elastase, collagenase and plasminogen activator
C Cationic proteins increase vascular permeability and cause chemotaxis
C Neutral proteases degrade extracellular matrices

OXYGEN-DERIVED FREE RADICALS
Hydrogen peroxide (H$_2$O$_2$)
Superoxide anion (O$_2^-$)
Hydroxyl radicals (OH$^-$)
Produce:
* Endothelial cell damage with resultant increased vascular permeability.
* Inactivation of antiproteases, thus leading to unopposed protease activity, with increased destruction of ECM.
* Injury to a variety of cell types (tumour cells, red cells, parenchymal cells).

NOTE: Oxygen metabolites are detoxified by antioxidants. Antioxidants include the serum proteins ceruloplasmin and transferrin and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The net effects on tissue injury of oxygen metabolites depend on the balance between their production and inactivation.

SUMMARY OF MEDIATORS IN INFLAMMATION

<table>
<thead>
<tr>
<th>EVENT</th>
<th>MEDIATOR OR MECHANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasodilation</td>
<td>Histamine, bradykinin, prostaglandins-I$_2$, E$_2$, D$_2$, F$_2^*$, Nitric oxide</td>
</tr>
<tr>
<td>Increased vascular permeability</td>
<td>Histamine, serotonin, bradykinin, fibrinopeptides, C3a and C5a (anaphylatoxins), leukotriene C$_4$, D$_4$, E$_4$, PAF, oxygen metabolites</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>LTB$_{4}$, C5a, bacterial products, neutrophil cationic proteins, cytokines - chemokines (IL-1, TNF, IL-8), PAF</td>
</tr>
<tr>
<td>Fever</td>
<td>IL-1, IL-6, TNF, prostaglandins</td>
</tr>
<tr>
<td>Pain</td>
<td>PGF$_{2\alpha}$, bradykinin</td>
</tr>
<tr>
<td>Tissue damage</td>
<td>Oxygen free radicals, lysosomal enzymes, Nitric oxide</td>
</tr>
</tbody>
</table>