ACUTE INFLAMMATION

FUNCTION OF THE LOCAL EVENTS OF INFLAMMATION

- Move defense mechanisms from vascular system out to tissues
- Results in several basic mechanisms activated at once
  1. Increased vascular permeability
  2. Leukocyte production
  3. Chemotaxis and phagocytosis
  4. Coagulation
  5. Neovascularization
  6. Fibrinolysis
  7. Fibroplasia and repair

2 MAJOR COMPONENTS OF THE INFLAMMATORY RESPONSE

1. Vascular Changes
   - Vasodilation (change in caliber and flow)
   - Increased Vascular Permeability – Acute Local Active Hyperemia

2. Cellular Events
   - Movement from capillaries and post capillary venules (emigration)
   - Accumulation of leukocytes at sites of injury (migration)
   - Activation of inflammatory cells and removal of stimulus

SEQUENCE OF EVENTS IN ACUTE INFLAMMATION

1) Vasodilation
   a) Arteriolar Dilation (sometimes see vasoconstriction first – neurogenic)
      i) Result of Histamine and Nitrous Oxide (primarily)
      ii) Increases the amount of blood to tissue (blood volume)
      iii) Opens additional capillaries (previously not open)
         (1) Produces the redness and heat seen in acute inflammation
   b) Dilation of capillaries and venules
2) Increase permeability of microvasculature – outpouring of fluids into extravascular tissues
   a) Discussed more thoroughly on the next page
   b) Postcapillary venules are important sites
3) Concentration of erythrocytes in capillaries and veins is increased because of the outflow of fluid
4) Blood flow slows (stasis)
5) Margination of WBC in capillaries and venules
6) Pavementing of WBC in capillaries and venules
   a) Result of upregulation of adhesion molecules on endothelial cells and WBC’s
7) Exudation of WBC

Permeability Changes - Vascular Permeability (Vascular Leakage)

Transudates were discussed in circulatory disturbances. Transudates are the result of increased hydrostatic pressure or decreased colloid osmotic pressure in the vasculature. The fluid which enters the extravascular space has low protein and cellular contents. Fluids with more protein and more white cells are found in extravascular spaces when endothelial gaps are opened or there is endothelial cell damage. The following chart may be helpful to differentiate the two. The term modified transudate is used when the fluid does not have “true” characteristics of either transudate or exudate. The fluid seen in Feline Infectious Peritonitis is a good example of a modified transudate as it tends to be protein rich and cell poor.
TRANSUDATE VERSUS EXUDATE

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>EXUDATE</th>
<th>MODIFIED TRANSUDATE</th>
<th>TRANSUDATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEFINITION</td>
<td>Inflammatory</td>
<td>-</td>
<td>Non-inflammatory</td>
</tr>
<tr>
<td>ETIOLOGY</td>
<td>Inflammation Infection</td>
<td>Early or late inflammation, hydrostatic psi or vascular permeability</td>
<td>Non-inflammatory edema</td>
</tr>
<tr>
<td>SPECIFIC GRAVITY</td>
<td>Above 1.025</td>
<td>1.017-1.025</td>
<td>Below 1.017</td>
</tr>
<tr>
<td>PROTEIN CONTENT</td>
<td>More than 30 g/L</td>
<td>25-75 g/L</td>
<td>Less than 25g/L</td>
</tr>
<tr>
<td>CLOTTABLE</td>
<td>Often</td>
<td>Rarely</td>
<td></td>
</tr>
<tr>
<td>INFLAMMATORY CELLS</td>
<td>Usually</td>
<td>Few</td>
<td>Occasionally</td>
</tr>
<tr>
<td>BACTERIA</td>
<td>Usually</td>
<td>Few</td>
<td>Occasionally</td>
</tr>
</tbody>
</table>

First plasma ultrafiltrate - due to small interendothelial gaps.............[Transudate]
More protein in fluid when permeability increases............................[Exudate]
Responsible for clinical signs of swelling

The hallmark of acute inflammation is increased vascular permeability. Normal fluid exchange and microvascular permeability are dependent on an intact endothelium. Five mechanisms of increased vascular permeability are described:

1. **Formation of endothelial gaps in venules**
   (a) Endothelial cell contraction - Rapid
   Œ Widening of intercellular junctions (gaps)
   Mediators: histamine, bradykinin, leukotrienes
   Immediate Transient Response
   Binding of mediator to receptor Œ contraction
   Short lived 15 - 30 minutes
   Reversible
   Affects only venules 20 to 60 : m in diameter
   (Capillaries and arteries not affected)
   (b) Endothelial retraction – Delayed and prolonged
   Cytoskeletal and junctional reorganization
   Reversible
   Structural reorganising of cytoskeleton - disruption of endothelial junctions
   Starts 4-6 hours lasts and lasts 24+ hours
   Mediators: tumor necrosis factor (TNF), interleukin-1 (IL-1), Interferon-gamma (IF-γ)

2. **Direct endothelial injury**
   Immediate Sustained Response
   Arterioles, venules and capillaries affected
   Lasts for several hours to days until vascular structures are repaired or thrombosed
   Causes: Damage directly to endothelium
   Eg: severe burns or lytic bacterial infections
   Milder damage - delayed prolonged leakage (2 to 12 hours)
   Eg: some toxins, thermal injury
   Result of apoptosis (?)

3. **Leukocyte dependent endothelial injury**
   Cause: WBC’s aggregate and adhere to endothelium
   - Become activated - release toxic oxygen species and proteolytic enzymes, which then cause endothelial injury or detachment - resulting in increased permeability.
   Sites – venules – sites where neutrophils can adhered
   Time - late response

4. **Increased transcytosis**
   - Transport of fluid through endothelial cells by channels of interconnected, uncoated vesicles and vacuoles (vesiculovacuolar organelles).
   - Certain factors (vascular endothelial growth factor) can increase the number and size of these channels. May be important method used with histamine and other chemical mediators

5. **Leakage from regenerating capillaries**
   Cause: Proliferating endothelial cells are leaky
   Time: Seen in repair process
   Mediators: VEGF (vascular endothelial growth factor)
Mediators of Vascular Permeability

(These mediators will be discussed in later lectures – but may be handy to know a little about them now…)

<table>
<thead>
<tr>
<th>Types of Mediator</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoactive Amines</td>
<td>Histamine and serotonin</td>
</tr>
<tr>
<td></td>
<td>- Stored in mast cells, basophils and platelets as granules</td>
</tr>
<tr>
<td>Plasma kinins</td>
<td>Bradykinin (principle vasoactive amine)</td>
</tr>
<tr>
<td></td>
<td>- Generated from plasma precursors by enzymatic cleavage</td>
</tr>
<tr>
<td>Complement Fragments</td>
<td>C5a and C3a</td>
</tr>
<tr>
<td></td>
<td>- Work indirectly by causing WBC’s to release mediators</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>LTC4, LTD4, LTE4 (independent of neutrophils)</td>
</tr>
<tr>
<td></td>
<td>LTB4 – works dependently via neutrophils</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>PGE2 and PG12 – vasodilation &amp; potentiates vascular leakage</td>
</tr>
<tr>
<td></td>
<td>TXA2 – causes vascular leakage</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Interleukin 1 (IL-1)</td>
</tr>
<tr>
<td></td>
<td>Tumour necrosis factor (TNF)</td>
</tr>
<tr>
<td>Platelet Activating Factor (PAF)</td>
<td>Derived from WBC’s</td>
</tr>
<tr>
<td></td>
<td>Induce “Second Phase”</td>
</tr>
<tr>
<td></td>
<td>Works on endothelium and WBC’s</td>
</tr>
</tbody>
</table>

**COMPLEX HUMORAL SIGNALS ARE RELEASED FROM DAMAGED TISSUES.**

**Tumor necrosis factor** (TNF) and **interleukin 1** (IL-1) are major cytokines that mediate inflammation. Not only due they act locally, but there are affects are systemic and produce the “acute-phase responses” associated with infection or injury. These responses include fever, loss of appetite.

Acute phase response to inflammation
- Includes many physiological adaptive changes
  - Fever, anorexia, CNS depression, slow-wave sleep, the release of corticotropin and corticosteroids, and promote lipid and protein mobilization.

- Characteristic leukocyte and plasma protein changes

Bone Marrow and Lymphoid Tissue
- Alterations in the rate of production and release of leukocytes.

Liver
- Production of specific "acute phase proteins" by hepatocytes in response to inflammation (IL-1 and TNF) and certain stressors (neoplasia and toxins).

  - C-reactive protein
  - Serum amyloid A
  - 2-macroglobulin
  - Haptoglobin
  - Ceruloplasmin
  - Fibrinogen

(Interestingly transferrin is decreased in concentration and synthesis of albumen is decreased when acute phase proteins are synthesized.)
CELLULAR EVENTS
In order to understand the cellular events in inflammation, the major characteristics of inflammatory cells must first be discussed.

CELLS OF THE INFLAMMATORY EXUDATE
Polymorphonuclear Leukocytes (Synonym: granulocytes)
- Neutrophils
- Eosinophils
- Basophils / Mast Cells

Mononuclear cells
- Lymphocytes and plasma cells.
- Monocytes and Macrophages
- Platelets

GENERALITIES
1. Most are normal inhabitants of the circulating blood (exceptions: plasma cells, macrophages, and mast cells).
2. The total leukocyte count (WBC) in peripheral blood and the relative proportions of different white blood cells may be greatly modified in systemic response to inflammation.
3. Each cell type plays a fairly distinctive role.
4. Each cell type enters into the inflammatory response in a definite sequence.

POLYMORPHONUCLEAR CELLS - GRANULOCYTES

NEUTROPHILS (Synonyms: polymorphs, Polys, PMN's, Neuts)
Characteristics
- High motility due to rapid amoeboid movement
- Respond to a wide variety of chemotactic compounds
- Phagocytic and bactericidal activities
- Neutrophils are the major cellular defense system against bacteria
- Are a major part of the innate immune system - 1st line of defense
- Crucial to the entire inflammatory process
- Neutrophils have surface receptors for complement fragment C3b and Fc portion of immunoglobulin
- End cell – don’t divide

2 distinct pools of neutrophils in the blood:
1. Marginating Pool: Neutrophils within blood vessels but lying out of the flow -or- "marginated" against the walls.
2. Circulating Pool: Neutrophils in circulation
   a. T½ ~ 4-6 hours
      - Circulating and marginating pools are approximately equal in size
      - Neutrophils in the marginating pool can be mobilized very quickly
      - Once neutrophils go out of the vasculature they do not return
      - Live 1-2 days in tissue.
   b. 2 major sources of reserve neutrophils are
      1. marginating pool
      2. bone marrow
Morphology of neutrophils:
- 10-12 µm in diameter with a multilobed nucleus.
- Contain abundant cytoplasmic granules.
  Several (up to 5) classes and subclasses have been identified
  
  **Azurophil Granules** (primary granules)
  - large, oval and electron dense
  
  **Specific Granules** (secondary granules)
  - smaller, less dense and more numerous
  
  **Tertiary granules** (gelatinase granules)
  
  Note: Differentiating neutrophils from eosinophils in rabbits, guinea pigs, rats, reptiles, fish and birds is difficult because the neutrophils have prominent eosinophilic granules and are difficult to differentiate from eosinophils. They tend to be grouped together,

### NEUTROPHIL GRANULE CONSTITUENTS

<table>
<thead>
<tr>
<th>AZUROPHILIC GRANULES</th>
<th>SPECIFIC GRANULES</th>
<th>TERTIARY GRANULES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} granules</td>
<td>2\textsuperscript{nd} granules</td>
<td>Gelatinase granules</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>Collagenase</td>
<td>Gellatinase</td>
</tr>
<tr>
<td>Elastase</td>
<td>Lysozyme</td>
<td>Lysozyme</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Histaminase</td>
<td>CD11b/CD18 \textsuperscript{$} integrin</td>
</tr>
<tr>
<td>Cathepsin G and others</td>
<td>Heparinase</td>
<td>CD11b/CD18 \textsuperscript{$} integrin</td>
</tr>
<tr>
<td>Proteinase-3</td>
<td>Cytochrome \textit{b}</td>
<td>Urokinase plasminogen activator receptor</td>
</tr>
<tr>
<td>\textit}{$\alpha$}-glucuronidase</td>
<td>Vit B\textsubscript{12}-binding protein</td>
<td></td>
</tr>
<tr>
<td>\textit}{$\beta$}-mannosidase</td>
<td>TNF-\textit{$} receptor</td>
<td></td>
</tr>
<tr>
<td>Defensins</td>
<td>CD11b/CD18 \textsuperscript{$} integrin</td>
<td></td>
</tr>
<tr>
<td>Bactericidal/permeability-increasing protein</td>
<td>urokinase plasminogen activator</td>
<td></td>
</tr>
<tr>
<td></td>
<td>activator</td>
<td></td>
</tr>
<tr>
<td></td>
<td>urokinase plasminogen activator receptor</td>
<td></td>
</tr>
</tbody>
</table>

Function:

**Phagocytosis**
Ingest, neutralize, and kill/destroy ingested material

**Killing mechanisms**
a. Production of oxygen free radicals
b. Hydrogen peroxide
c. Lysosomal enzymes

**Mediate tissue injury**
via release of oxygen free radicals and lysosomal enzymes

**Regulate** inflammatory response
via releasing chemical mediators
Leukotrienes
Platelet activating factor
EOSINOPHILS

**Characteristics**

Numerous at inflammatory sites which result from
- Parasites
- Allergic or Immunologic Disease
- Some fungi

May be present in any exudate
- 1-5% WBC

Phagocytic but less so than neutrophil

Present in tissues in contact with environment
- Intestine
- Skin
- Mucous membranes
- Lung

Sensitive to corticosteroid therapy

Cytokines important for production
- IL-3, IL-5 and GM-CSF

Ratio of eosinophils
- blood: bone marrow: tissue
  - 1:200:500

**Morphology**

- Granules vary in size (dependent upon species)
- Granules stain with acid dye eosin - hence their name
- Slightly larger than neutrophils
- Lysosomal granules contain a wide variety of catalytic enzymes similar to those in neutrophils, except they do not contain lysozyme
- Antiparasitic proteins present in granules include
  - Major basic protein
  - Eosinophil cationic protein

**Function**

Work to kill or damage helminths and other pathogens

Cause and assist in hypersensitivity reactions
  - Especially Type I hypersensitivities

Regulator of inflammation - particularly to mast cell products

Killing helminths by antibody-dependent cell-mediated cytotoxicity

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**DISTINCTIVE CHARACTERISTICS OF EOSINOPHILS**

<table>
<thead>
<tr>
<th>CONSTITUENT OR PRODUCT</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major basic protein</td>
<td>Parasite killing</td>
</tr>
<tr>
<td></td>
<td>Induces histamine release from mast cells</td>
</tr>
<tr>
<td></td>
<td>Neutralize heparin from mast cells</td>
</tr>
<tr>
<td>Eosinophilic cationic protein</td>
<td>Parasite killing</td>
</tr>
<tr>
<td></td>
<td>Shortens coagulation time</td>
</tr>
<tr>
<td></td>
<td>Alters fibrinolysis</td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>Inactivates leukotrienes (LTC4, LTD4, LTE4)</td>
</tr>
<tr>
<td>Histaminase</td>
<td>Inactivates histamine</td>
</tr>
<tr>
<td>Phospholipase D</td>
<td>Inactivates platelet-activating factor</td>
</tr>
</tbody>
</table>
BASOPHILS AND MAST CELLS

Characteristics:
- Basophils are rare circulating granulocytes
- Mast cells are found in perivascular sites
- Both derived from bone marrow
- Contain abundant cytoplasmic metachromatic granules
  1. Metachromatic granules stain pink to blue with toluidine blue
  2. Result of high content of sulphated mucopolysaccharides
- 1° heparin
- Granules also contain histamine, proteases, + potent inflammatory mediators
- Receptors that bind the Fc portion of IgE antibody
- Major source of histamine - acute inflammation
- Produce cytokines
  - TNF-", IL-1,-3,-4,-6-,-8, IFN-(
- Major cellular mediator of Immediate Hypersensitivity Reactions (Type I)
- Don’t die after release of granules

Morphology:
- Mast cells - round nuclei with abundant cytoplasm filled with granules
  - Found in connective tissue in perivascular spaces
  - Contact with environment - (lung, gut, mm, skin)
  - 2 subtypes
    - Mucosal mast cells: seen in gastrointestinal and respiratory tract
    - Connective tissue mast cells: found in the skin
- Basophils – from blood and multilobed nuclei
  - Are recruited to sites in hypersensitivities

Functions:
- Intimately involved in acute inflammation
  - Release of histamine
  - smooth muscle contraction
  - vascular permeability
- Involved in recruitment of Eosinophils
  - Cause other cells to secrete eotaxins
- Generate Cytokines

MACROPHAGES/ MONOCYTES

Characteristics:
- Macrophages:
  - Derived from circulating blood monocyte of bone marrow origin
  - Some originate from immature resident mononuclear phagocytes
  - "Histiocytes" another name for tissue macrophages
- Monocytes:
  - Do not have a large reserve pool in the bone marrow
  - Remain longer in circulation, (t ½ 24-72 hours)
  - Are functional cells but require activation to become macrophages
    - Various chemical mediators
    - Monocytes migrate into tissues and then are called macrophages
    - Motile - but sluggish
    - T ½ 30-60 days but can proliferate

Morphology:
- Larger (15-20 : m) than neutrophils
- Prominent, usually central nuclei, which may be folded or bean-shaped
- Contain many lysosomes and have cytoplasmic extensions

Function:
- "Most dynamic and gifted of the leukocytes." (box 4-3 page 177)
  - Antimicrobial and phagocytic (Oxygen radicals)
  - Recruit other Leukocytes (Chemokines and Cytokines)
  - Stimulate or modulate other cell activity (Vascular effects)
Clean up debris
Induce systemic effects
Source of multinucleated giant cells and epithelioid cells

**LYMPHOCYTES AND PLASMA CELLS** (review Immunology notes)

**Characteristics:**
- Principally involved in immune reactions
- Immediate antibody response
- Delayed cellular hypersensitivity responses
- Less motile than neutrophils and monocytes
- Plasma cells produce and release antibody (originate from B cells)
  -Produced by 1° lymphoid organs
  -Migrate to 2° lymphoid tissue (spleen, lymph node)
-Recirculate

**Morphology:** Heterogeneous in size and morphology
Function heterogeneity (T cells and B cells)
- Subclasses of lymphocytes express different cell surface proteins
  - eg. CD4 helper T cells
  - eg. CD8 (cytotoxic) suppressor T cells
- Smaller than neutrophils, dense staining nucleus

**Function:**

**B lymphocytes:**
- Important in production of antibody (humoral immunity)
- Antibody constitutes one of the major opsonins
  Interfaces directly with cellular and phagocytic arms of host defence mechanism.

**Lymphocytes:**
- Responsible for cell mediated immunity
- TH1 lymphocytes produce lymphokines
  - Modulate and expand local inflammatory reactions
    - IFN-γ, TNF-α, IL-2

**PLATELETS AS INFLAMMATORY CELLS**

**NOTE:** In addition to their role in hemostasis and coagulation, platelets are very important in inflammation. Primary hemostasis is a part of the inflammatory response.

Products from activated and/or aggregated platelets:
- Fibrinogen
- Coagulation factors VIII and V
- Histamine
- Ca++ cations
- Complement-cleaving proteases
- Growth factors

Contributions to the inflammatory response
- Release constituents that increase vascular permeability
- Release constituents that may provide local amplification
- Release cationic inflammatory mediators
- Release enzymes that can directly activate C5
- Chemotactic activity for leukocytes

Let’s not forget endothelial cells and fibroblasts...