

## MEDIATORS OF INFLAMMATION

Reading: Basic Pathology, Chapter 2, pp 44-59.

Summary: Chemical mediators and cellular mechanisms that are involved in the development of the inflammatory response are described in some detail. The mechanisms for the systemic responses to inflammation are also discussed.

### I MECHANISMS IN INFLAMMATION - a review

#### A. General Characteristics

1. Heat, redness, pain, swelling, loss of function.
2. A reversible change.
3. Constant nature; orderly sequence of events.
4. Seen only in living, vascularized tissues.
5. Latent period between insult and reaction.
6. Suppression by drugs - antihistamines, steroids, aspirin, etc
7. .

#### B. Basis of reaction - vascular phase in arterioles and venules.

1. Vascular response. Vasodilatation. Physiological permeability gives way to pathological increase in permeability; protein into tissues.
2. Structure of microvasculature and changes - desmosomes separate; loss of continuity in endothelium; passage of particles to basement membrane.
  - a. Sieving by membrane.
  - b. Some exudation due to increased filtration pressure and direct vascular injury.

### II ROLE OF CHEMICAL MEDIATORS OR AUTOCOIDS (local hormones).

#### A. These are important because they are:

1. Endogenous substances causing changes seen in inflammation.
2. Produced, released, or activated by injury.
3. Present and active during inflammation but absent or inactive at other times.
4. Inhibited by specific natural or synthetic antagonists inhibiting various phases of inflammation.

#### B. Important chemical mediators that give rise to an intense local activity, distinguished from neurotransmitters by the site of action and from hormones by the local nature of the response.

1. Plasma factors - proteases mostly.
  - a. Kinins
  - b. Complement factors (C3a, C5a, C567)
  - c. Coagulation system - fibrinolytic system
2. Cell Products
  - a. Vasoactive amines (histamine, serotonin).
  - b. Neutrophil products
  - c. Cytokines
3. Lipid mediators. Phospholipids of the cell membrane - most released by phospholipase A<sub>2</sub> action on arachidonic acid.
  - a. Prostaglandins (PG).
  - b. Leukotrienes (LT)
  - c. Platelet activating factor (PAF)
  - c. Lipoxins

#### C. Amines - principal mediators of early phase of inflammation.

1. Histamine - stored in mast cells, basophiles, and platelets; the latter contain serotonin that has activities similar to histamine: vasodilatation, increased vascular permeability in venules, and contraction of smooth muscles. Histamine is the only preformed mediator available in large amounts.

- a. Release with trauma, immunological reactions, C3a and C5a, cytokines, IL-1, & -8, and cationic proteins from PMNs.
  - b. Release by fusions of cytoplasmic granules, then fusion with plasma membrane.
  - c. Causes arteriolar dilation and increases venular caliber and permeability. H<sub>1</sub> receptors on inflammatory cells and vessels are involved in increased vascular responses.
  - d. Level decreased in about 1 hour. Antihistamines counteract many aspects of the early inflammatory reactions.
- D. Plasma factors - Kinins, complement, & blood clotting factors.
1. Kinin system - results in production of peptide, bradykinin:
- a. Increases vascular permeability, potent dilator of venules, causes pain.
  - b. Destroyed by kininase.
  - c. Activated by proteases, kallikrein, and Factor XII that have additional functions. Kallikrein is in plasma and tissues. Factor XII (Hageman factor) is also important in blood coagulation. Activated by contact and split to form factor XIIA. Also important in fibrinolysis system.

plasmin, kallikrein, contact

step 1. factor XII-----> factor XIIA

factor XIIA

step 2. prekallikrein----->kallikrein

kallikrein

step 3. kininogen----->bradykinin

2. Complement system - series of factors originally described as interacting with antibody - antigen complexes to mediate immunological injury. Mechanism of action: Classical pathway activated via C1, C4 and C2; alternative pathway activated via C3, Factors B and D. Most important factors with respect to inflammation are C3a, C5a, and C5b-9 (MAC).
- a. Activators of alternative pathway: immune complexes (Fab fraction of Igs), microorganisms, fungi, enzymes, and tissue products.
  - b. C3a and C5a give rise to histamine release and thus increased vascular permeability. C5a stimulates release of leukotrienes from neutrophils and macrophages.
  - c. C5a is a chemotactic factor for PMNs & mast cells.
  - d. C5b-9 important in cell lysis.
  - e. C3b products are also important as opsonins.

The complement system is taken up in more detail in Microbiology.

3. Coagulation factors - clotting and fibrinolytic systems.
- a. Plasminogen split to give plasmin (a protease) - a clot lysis factor.
  - b. Plasmin also activates factor XII to XIIA yielding bradykinin and cleaves C3 to yield C3a.
  - c. Finally, split fibrin and fibrinopeptides are factors increasing vascular permeability and chemotaxis for PMNs.
  - d. Protein C. Promotes fibrinolysis, inhibits thrombosis and inflammation.
4. Summary of important serum factors.
- a. Bradykinin, C3a and C5a cause increased permeability(the latter two indirectly by releasing histamine); C5a is a

- chemotactic factor, and bradykinin gives rise to pain.
- b. C3a and C5a are generated from cleavage of C3 and C5 by several mechanisms: plasmin & contact.
- c. Factor XIIA important in the clotting, fibrinolysis, complement, and kinin systems. Kallikrein, factor XII, and plasmin all act to catalyze XIIA formation.

E. Eicosinoids - lipid mediators

1. Prostaglandins (PG) and thromboxane A<sub>2</sub> (Tx A<sub>2</sub>) - derived by cyclooxygenase (COX) pathway from arachidonic acid, that is also the precursor of leukotrienes. At least three forms of COX exist, COX-1, 2, & -3. COX-2 appears specifically in inflammation. Various cells produce different PG's and TxA<sub>2</sub>, PGE, thromboxane, prostacyclin, etc. Receptors are for most part G protein-coupled.
  - a. Almost all cells produce PGs. They are especially important in inflammation, but present in exudates 6-24 hours later than histamine and bradykinin; so, responsible for later effects than these. PGs are not stored - produced de novo.
  - b. PGE<sub>2</sub> gives rise to vasodilatation and potentiates effects of histamine and other mediators in increasing vascular permeability.
  - c. PGE, like bradykinin, can induce pain.
  - d. PGE induces fever, an effect countered by aspirin.
  - e. In short, PGs alone or by potentiating other mediators do everything except attract leukocytes. They increase blood flow and cause pain and fluid leakage.
  - f. Prostacyclin (PGI<sub>2</sub>) potent vasodilator. Inhibits platelet aggregation. Made by endothelial cells and macrophages.
  - g. Thromboxane - platelet product causing platelet aggregation/activation and vasoconstriction. Enhances function of inflammatory cells by increasing cyclic GMP levels.
  - h. Aspirin irreversibly inhibits PG synthesis by inhibiting COX-1 & -2 activity so that arachidonic a. can't be oxidized to yield PGE or PGF. Blocking the action of COX-1 gives rise to stomach damage, disrupts renal salt and water balance, & platelet ability to aggregate. Newreversible COX-2 specific inhibitors do not have these activities.
  - i. COX-3: Present in brain tissue. Blocked by acetaminophen (Tylenol)
2. Leukotrienes (SRS-A) produced by the lipoxygenase pathway. Steroids inhibit the formation of both LTs and PGs by decreasing the supply of arachidonic a. through inhibiting the activity of the enzyme phospholipase A<sub>2</sub>. LTs activated by immune complexes and bacterial products. LTs use G protein-coupled receptors.
  - a. Active late in inflammatory response.
  - b. Increase venular permeability at very low concentrations (most potent mediator). Can work with PGE.
  - c. Act as powerful chemotactic and cell adhesion factor for eosinophils and PMNs (LTB<sub>4</sub>). Cause bronchospasm - important in asthma. Development of new anti-LT drugs for asthma therapy.
  - d. Facilitates release of lysosomal enzymes from PMNs.
3. Lipoxins - made by collaboration between PMNs & platelets
  - a. Inhibit PMN activity by decreasing poly recruitment, chemotaxis, and adhesion to endothelial cells; stimulate monocyte adhesion.
  - b. Vasodilators

- F. Platelet activating factor (PAF) - a phospholipid from basophiles, PMNs, monocytes and macrophages inducing:
  1. Release of mediators (esp. PGs, LTs, IL-1, TNF, and IL-6)
  2. Activation of PMNs
  3. Increased vascular permeability, vasodilatation, and pain. Ischemotactic, induces platelet aggregation and induces platelet aggregation and degranulation, degranulation of PMNs and bronchoconstriction.
- G. Neuropeptides
  1. Substance P - important in pulmonary & G.I. inflammation. Induces increased vascular permeability and smooth muscle contraction and transmission of pain signals. Produced for instance by pulmonary branches of vagus nerve.
  2. Peripheral opioid production. Controls pain. Counteracted by substance P.
- H. Cytokines - important as immunological and growth factors. Termed cytokines as a group (see table).
- I. Nitric oxide (NO) - a soluble gas that is a potent vasodilator due to relaxation of smooth muscle. Made both constitutively (eNOS, nNOS) & induced (iNOS) in cytokine-activated macrophages.

NO is discussed more fully in Microbiology.

- III. Inhibition of any phase of leukocyte function or a serious leukopenia gives rise to chronic, serious bacterial infections. Examples: Chronic Granulomatous Disease due to defect in NADPH-oxidase, causing eventually lack of PMN hydrogen peroxide. Chediak-Higashi syndrome, a defect in formation of poly granules. Leukocyte adhesion deficiency.
- IV. Summary of mediators - there is a high degree of redundancy in the inflammatory response - many factors do the same thing because it's important that the job get done. Rapid destruction of mediators important.
  - A. Key mediators in phases of inflammation.
    1. Vasodilatation - PG's, nitric oxide.
    2. Increased vascular permeability.
      - a. Early - histamine and C3a and C5a by giving rise to histamine.
      - b. Late - in decreasing order of potency LTs, bradykinin, substance P, & PAF.
    3. Leukocyte binding - PAF, IL-8, C5a, TNF- $\alpha$  activation of  $\beta$  integrins CD11 and CD18.
    4. Chemotaxis: C5a, IL-8, bacterial products, PAF, & LTB<sub>4</sub>.
    5. Fever - PG's, IL-6, TNF- $\alpha$  and IL-1.
    6. Pain - PG's and bradykinin.
    7. Tissue damage - lysosomal matrix metalloproteinases (MMPs) from PMN's and monocytes, free oxygen radicals, and nitric oxide.
- V. Systemic Effects of Inflammation. Can be induced by TNF, IL-1 & IL-6. In addition, CRF, neuropeptides such as Substance P in the lung, PAF, & PGs play a role. Coordinated by nuclear factor- $\kappa$ B (NF- $\kappa$ B).
  - A. Fever - due to PG's, IL-1, IL-6, & TNF.
    1. IL-1 & TNF, produced by monocytes and macrophages, act directly on the vasomotor center and also induce PGE production the hypothalamus.
    2. PGE induces fever when injected into the ventricular system. PGE<sub>2</sub> present in CSF during fever. Aspirin decreases fever due to increased PGE synthesis.
  - B. Leukocytosis-especially in bacterial diseases.
    1. White count greater than 10,000 per mm<sup>3</sup>.
    2. "Shift to left" - immature leukocytes in circulation due to proliferation of bone marrow and rapid release of cells.

3. Can also have lymphocytosis, eosinophilia, - or leukopenia with different infections.
- C. Increase in erythrocyte sedimentation rate (ESR). Depends on ratio serum protein to RBC.
- D. Acute phase proteins produced in inflammation such as the antibacterial C-reactive protein (CRP) & serum amyloid A (SAA) that gives rise to secondary amyloidosis. CRP as predictor of coronary artery disease suggests importance of inflammation in this condition.
- E. Activated protein C is an endogenous substance that promotes fibrinolysis & inhibits thrombosis and inflammation that is now employed to treat severe infections.
- F. Release of enzymes from dying cells. Specific isoenzyme elevation with specific damage to a particular organ.
- G. Coordination of inflammation at cellular level by nuclear factor NF- B, the production of which is activated through the Toll-like receptor family (TLR) pathway, and inhibited by steroid treatment. TLRs are also discussed in Dr. Snapper's and in Microbiology lectures

Remember - inflammation can be both good and bad; removal of inciting agents and preparation for repair vs. tissue damage and scarring resulting in permanently impaired function. Apoptosis is not associated with an inflammatory response for a good reason - tissue damage associated with inflammation is obviated in apoptosis.