INFLAMMATION

This pdf was developed by
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Note on References

See box to the right for reference notations.

PubMed references usually link to abstracts, which provide useful information. But most abstracts link to a free, full-text article for in-depth reading.

Image references usually link to a whole page of images. The images themselves may be useful, but care must be taken to ensure that they relate to the topic of the text. Images are most valuable as a gateway to publications related to the topic. Clicking on the image will take you to the publication. However, to get the full publication it sometimes may be necessary to copy the url and transfer it to your browser.

- **Reference notations: when references are listed, the following suffixes indicate:**
- **pm = PubMed**
- **w = Wikipedia**
- **i = Images (usually Google images)**
- **rg = ResearchGate**
- **yt = YouTube**
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- **ow = Other website**
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What is inflammation?

- Inflammation is the body's response to injurious agents.
- Its "purpose" is to rid the body of the cause of the injury or to contain it so as to mitigate the extent of its damage.

What are the types of inflammation?

- **Acute inflammation: short term response to tissue injury.**
- **Chronic inflammation: longer term response to tissue injury.**

What are the pros and cons of inflammation?

- **Pros: contains and destroys injurious agent(s)**
- **Cons: secondary damage to tissues**

Acute Inflammation

Acute Inflammation: Tissue Injury

What are the causes of acute inflammation?

- When looking for the cause of inflammation, one or more of these will FIT IT!
- Foreign body presence
- Infection
- Trauma

- Immune reaction
- Tissue necrosis

What are the causes of acute inflammation?

- When looking for the cause of acute inflammation, one or more of these will FIT IT!
- **F**oreign bodies (e.g., dirt, sutures, various crystals)
- **I**nfection (bacterial, viral, fungal, parasitic, microbial toxins)
- **T**rauma (eg.,burn injury)
- **I**mmune reaction (allergies, autoimmune disease)
- **T**issue necrosis (e.g., myocardial infarction)

Acute Inflammation: Innate Immune System

What initiates inflammation?

- Tissue injury (FIT IT): e.g., pathogens, cell damage, irritants
- Recognition of injury by the innate immune system.
- Activation of inflammatory responses by the innate immune system.

What is the innate immune system?

- Innate immune system is an inborn biological system that recognizes molecular patterns of pathogenesis carried by injurious pathogens.
- This system initiates an inflammatory response "designed" to destroy the pathogen.

What are the components of the innate immune system?

- Physical epithelial barriers against invaders
- Phagocytic leukocytes
- Dendritic cells (1i): messengers between the innate and the adaptive immune systems
- Natural killer (NK) cells (1i): innate cytotoxic lymphocyte
- Circulating plasma proteins
  - Natural antibodies
  - Complement proteins

Who discovered dentritc cells?

Dendritic cells were discovered in 1972 by **Ralph Steinman** and **Zanvil A. Cohn** at the Rockefeller University.

Ralph Steinman received the Nobel Prize for this discovery in 2011.

How is an infection initially sensed?

- Through pattern recognition receptors (PRRs) in the innate immune system.
- This system senses molecular patterns on pathogens through pattern recognition receptors (PRRs).
- These receptors include:
  - Toll-like receptors
  - RIG-I-like receptors
  - NOD-like receptors
  - C-type lectin receptors.
  - Other pathways
- These receptors trigger signaling cascades that initiate the inflammatory process.

Who discovered toll-like receptors?

- Jules Hoffmann and Bruce Beutler, who received the Nobel Prize in 2011 for discovery of toll-like receptors. Read a summary of this discovery.

What is a PAMP?

- Pathogen-associated molecular pattern (connect to receptors on dendritic cells that PAMPs that activate innate immune responses).
- PAMPS are recognized by toll-like receptors (TLRs) and other pattern recognized receptors
- PAMPS include:
  - Bacterial lipopolysaccharides (LPSs) (endotoxins) recognised by toll-like receptor 4 (TLR4)
  - Bacterial flagellin (recognized by TLR5)
  - Lipoteichoic acid (recognized by TLR2)
  - Peptidoglycan (recognized by TLR2)
  - Nucleic acid variants normally associated with bacteria and viruses(recognized by TLR3)

What is a DAMP?

- Damage-associated molecular pattern
- **DAMPs consist of host molecules that can initiate and perpetuate a noninfectious inflammatory response molecules from damaged tissues.**
- **Examples of DAMPs include:**
  - Chromatin-associated protein high-mobility group box 1 **(HMGB1)**
  - DNA and RNA
  - S100 proteins
  - Purine metabolites
  - Mono and polysaccharides

What is the “end product” of the innate immune system that initiates acute inflammation?

- **Chemical (Inflammatory) mediators produced by innate immune cells**
- **Examples: vasoactive amines and peptides, eicosanoids, proinflammatory cytokines, and acute-phase proteins.**

Acute Inflammation: Acquired Immunity

What is acquired (adaptive) immunity?

- Acquired immunity represents immunological memory after an initial interaction with a specific pathogen.
- This leads to greater response to subsequent encounters with that pathogen.
- Acquired immunity is the basis of vaccination.
- It includes both humoral immunity and cell-mediated immunity.

Where are features of the acquired immune system?

- Specificity to particular pathogens
- Long-term memory of immunity (e.g., vaccination)
- Antigens or any substances that elicit the acquired (adaptive) immune response.
- B lymphocytes are activated to secrete antibodies (immunoglobulins).
- T lymphocytes mediate cellular immunity.

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How does the acquired immunity system initiate an inflammatory response?

- Antigen interaction with memory T cells (1sd) can initiate an inflammatory response.
- Complement bridges innate and adaptive immune responses and allows an integrated host defense to pathogenic challenges.
- The acquired immune system is under the control of the innate system.
- The acquired immune system is more flexible and definitive in destroying specific pathogens, compared to the innate immune system.

Acute Inflammation: Vascular Response

What are the responses of the microvasculature in acute inflammation?

1. Vasodilatation (greater blood delivery)
2. Allows greater permeability (for plasma proteins)
3. Allows migration of leucocytes into tissues

What are mechanisms for increased vascular permeability in acute inflammation?

1. Retraction of endothelial cells resulting in opening of interendothelial spaces
2. Endothelial cell necrosis and detachment.
3. Increased transport of fluids and proteins , through the endothelial cell (called transcytosis)

How do lymphatic vessels respond to acute inflammation?

1. Increased lymphatic flow
2. Inflamed lymph channels (lymphangitis = red streaks)
3. Lymph node enlargement (lymphadenitis)

Acute Inflammation: Leukocyte Responses

How are leukocytes recruited to attack injurious agents?

- **In response to pathogenic invasion, the larger leukocytes (chiefly neutrophils at this stage) accumulate along the vascular endothelium (*margination*).**
- **Marginated leukocytes roll along the endothelium.**
- **Leukocyte integrin proteins enter a high-affinity state, adhering to endothelium and then migrating through the vessel walls into extravascular tissues (*diapedesis*).**

How do leukocytes first adhere to vascular endothelium? (1ow)

- They first connect through receptors called *selectins.*
- Selectins come in three varieties:
  - E-selectin (CD62E)
  - P-selectin (CD62P)
  - L-selectin (CD62L),
- Adhesion is strengthened by another set of receptors call integrins (VCAMs and ICAMs)

How do leukocyte transmigrate through the endothelium?

- The first step is adhesion between leukocytes and endothelial cells in post capillary venules (1pm, 2i)
- Migration occurs between endothelial cells; in the process both leukocytes and endothelial cells undergo deformation (1pm).
- At the same time, the phagocytic properties of leukocytes are enhanced.

Acute Inflammation: Phagocytosis

How do leukocytes find their target site?

- Leukocytes find their target through chemotaxis, i.e., movement of cells in response to a concentration gradient in chemical mediators.
- *Chemotaxis* (1pm, 2pm, 3pm) is governed by various substances (bacterial products, cytokines, complement system components, and products of the lipoxygenase pathway of arachidonic acid metabolism), which causes leukocytes to move towards the area of infection/injury.

What are the mechanisms whereby leukocytes undergo phagocytosis?

- **Leukocytes have a multifaceted function in phagocytosis:**
  - **Activation of leukocyte (1a-i, 1b-i, 1c-i, 2i)**
  - **Recognition and attachment to injurious agent (1i)**
  - **Engulfment of target (1i, 2i, 3i)**
  - **Degradation of target (1i)**

- **For a humorous account of the travels of neutrophil, see "the adventures of neutrophil dog" at the end of this section.**

What dangers are presented by leukocytes in acute inflammation?

- Leukocytes can cause tissue injury.
- They can contribute to dangerous systemic reactions (sepsis) or contribute to development of chronic inflammation.

By what mechanisms may leukocytes cause tissue injury?

- Crosstalk between different cell types, mediators, cytotoxic agents appears to contribute to leukocyte-inflicted tissue damage.
- For example, leukocytes are activated to secrete various growth factors, chemokines and cytokines, complement components, proteases, nitric oxide, and reactive oxygen metabolites, all of which may play a role in tissue injury.

Clinical Signs of Inflammation

What are the cardinal signs of acute inflammation?

- **The signs of inflammation have been known to humanity since the first shaman treated their first patient. They were codified by the Romans in a list taught to every first-year medical student today:**
- **Rubor (redness)**
- **Tumor (swelling)**
- **Calor (heat)**
- **Dolor (pain)**
- **Functio Laesa (loss of function)**

What causes rubor and calor?

- **Tissue redness and heat are caused by inflammatory mediators released by tissue cells. They stimulate:**
  - **Arteriolar vasodilation**
  - **Increased blood flow**
  - **Engorgement of capillary beds**

What causes tumour (swelling of tissues)?

- **Interendothelial spaces widen, increasing vascular wall permeability and fluid accumulation in tissue.**
- **Direct endothelial injury may also cause leakage of fluid into tissue.**
- **With these two changes, protein-rich fluid moves into extravascular tissues.**
- **Red blood cells are concentrated and blood viscosity increases; small vessels are dilated, blood flows more slowly (stasis).**
- **Visible swelling (“tumour”) is seen.**

Patterns of Acute Inflammation

What are different patterns of acute inflammation?

- Serous: effusion of fluid poor in protein and white blood cells. Example: serous otitis media
- Fibrinous: effusion of fluid rich in protein but not white blood cells. Example: fibrinous pericarditis.
- Purulent: accumulation of fluid rich in protein, neutrophils and dead cells. Example: purulent otitis media
- Ulcer: excavation of the surface of an organ or tissue due to inflammation. Example: leg ulcer

What is the role of purulence in inflammation?

- Pus is a combination of protein-rich fluid and dead leukocytes. It results from inflammation, but specifically identifies the presence of bacterial or fungal infection.
- Many inflammations are not purulent, but pus always indicates inflammation

Historically, what was meant by “laudable pus”?

- The term “laudable pus” was used from ancient times through the American Civil War
- “Laudable pus” meant a thick creamy white or whitish-yellow purulence. Originally taken to mean that a wound was healing well.
- Why? Before we knew what bacteria were, we could see that infections with “laudable pus” (chiefly Staphylococcus aureus skin infections, tended even before antibiotics to be superficial and non-fatal. Necrotizing and gangrenous wounds are characteristic of more invasive and dangerous bacteria (beta-hemolytic streptococcus, clostridium perfringens, etc.). Observation demonstrated that thinner, foul-smelling wounds meant a poor prognosis.
- This is useful in clinical evaluation even today, as the first step towards identifying a bacterial infection.
- We do not, of course, try to induce “laudable pus” or leave the infection untreated.

What is sepsis?

- Sepsis is potentially fatal condition resulting from the body's response from injury (usually infection) in which the response damages the body’s own tissues and organs.
- The primary infection most often involves lungs, brain, urinary tract, skin, and abdominal organs.

What are signs and symptoms of sepsis?

- Fever
- Hypothermia
- Tachycardia/New A-fib
- Hypotension/shock
- Confusion/delirium
- Body fluid abnormalities
- Metabolic acidosis
- Respiratory alkalosis
- DIC/hemorrhage
- Organ failure

What is the *acute phase response* to an inflammatory stimulus?

- The *acute phase response* is a complex systemic reaction that helps to reestablish homeostasis and promotes healing.
- It is characterized by systemic reactions to cytokine release in response to toxic products (e.g. bacterial LPS). These reactions include:
  - Fever
  - Leukocytosis
  - Tachycardia
  - Acute phase reactants (e.g., C-reactive protein)

What are the systemic responses to inflammation?

- 1) Fever – particularly in response to infection.
- 2) Elevated plasma acute-phase protein levels

  - C-Reactive Protein (CRP) is the most common laboratory marker used.

  - Increased fibrinogen causes erythrocytes to stack (rouleaux formation) and speeds sedimentation, hence the use of ESR (erythrocyte sedimentation rate) as a lab marker for inflammation.
- 3) Leukocytosis
- 4) Tachycardia, rigors, somnolence, malaise
- 5) Septic shock (triad of disseminated intravascular coagulation, hypoglycaemia, and hypotensive shock) in response to severe infections.

What are the medical benefits of inflammation?

- **Inflammation is a crucial part of immune defenses.**
- **It works to eliminate the cause of tissue insult and clear out the necrotic tissues and cells resulting from that insult.**
- **Without inflammation, we would not be able to fight off infection and other causes of damage such as toxins.**

What are the dangers of inflammation?

- **Too strong a response; too prolonged; inappropriate activation of inflammatory response.**
- **Injury to normal tissues or processes while clearing the original insult.**
- **Excessive inflammatory response: denoted by SIRS (systemic inflammatory response syndrome); septic shock; anaphylactic shock; "cytokine storm" (the real killer of the 1918 flu epidemic, which is still a serious threat in more recent strains of flu).**
- **Failure to resolve; progression from acute to chronic inflammation;**
- **Development of auto-immune diseases in which a normal host tissue becomes a target of immune attack.**

So, if inflammation is a good thing, why do we use these so much?

Why are anti-inflammatories most often prescribed?

- **Anti-inflammatory drugs are among the most widely used general-purpose pain killers.**
- **These drugs include NSAIDs (aspirin, ibuprofen, diclofenac acid) and, in more serious cases, corticosteroids.**

What conditions hamper the inflammatory reaction and make the sufferer more vulnerable to infection?

- Antineoplastic chemotherapy
- Diabetes
- Genetic and acquired immunodeficiencies
- Neutropenia
- HIV/AIDS
- Malnutrition
- Splenectomy

Mediators of Inflammation

What are the major mediators of inflammation?

- Histamine
- Cytokines
- Eicosanoids
- Platelet activating factor
- Bradykinin
- Cell adhesion molecules

Mediator of Inflammation: Histamine

What is the most immediate inflammatory mediator?

- **Histamine, which is stored in pre-formed granules in mast cells and basophils, can be released immediately.**
- **Histamine is responsible for the arteriolar dilation and increased vasopermeability required for the inflammatory process.**
- **It is involved in all forms of inflammation, particularly hypersensitivity reactions (hence antihistamines to target allergic symptoms).**

Mediators of Inflammation: Cytokines

What are cytokines?

- Cytokines are a category of small proteins that mediate cell signaling and inflammatory pathways.

What are the categories of cytokines?

- Chemokines
- Interferons
- Interleukins
- Lymphokines
- Tumour necrosis factor

Cytokine Mediators of Inflammation: Chemokines

What are features of chemokines?

- Chemokines induce directed chemotaxis in nearby responsive cells
- Chemokines are peptides (mass approximately 8-10 kilodaltons)
- They can be either pro-inflammatory or anti-inflammatory (hemostatic)
- Inflammatory chemokines act as chemoattractants for leukocytes
- They are released by different cell types of both innate immune system and adaptive immune system.

Cytokine Mediators of Inflammation: Interferons

What are Interferons?

- Interferons are signaling proteins produced by cells in response to bacteria, viruses, tumours, and parasites.
- They trigger immune defenses by activating macrophages and natural killer cells, and by upregulating antigen presentation by enhancing expression of histocompatibility complex antigens
- They interfere with viral invasion of cells.
- Interferons can be divided into five groups: alpha, alpha-II (or omega), beta, delta (or trophoblast) and gamma.
- These are distributed into Type I and Type II interferons (see next slide).
- Interferon therapy can be used to treat various malignancies and hepatitis C.

What are characteristics of type I and type II interferons?

- Type I interferons bind to a cell surface receptor complex known as the IFN-α/β receptor.
- Type I interferons are produced by fibroblasts and monocytes in response to a viral infection.
- They bind to target cells which stimulate production of proteins that prevent viral replication.
- Type II interferon (IFN-γ, immune interferon) is released by cytotoxic T cells and T helper cells. It stimulates and modulates the immune system.

Why is type II interferon (IFN-γ) important?

1. IT stimulates Th0 (naïve) T-cells to differentiate to Type I T helper cells (Th1); suppresses Type II T helper cells (Th2) differentiation…positive feedback pro-inflammatory loop.
2. Promotes leukocyte adhesion, binding, and diapedesis.
3. Stimulates lysosome activity and antigen presentation in macrophages.
4. Activates inducible nitric oxide synthase.
5. Increases expression of class I Major Histocompatibility Complex (MHC) on cells in general, and class II MHC on antigen-presenting cells.
6. Induces granuloma formation through macrophage activation.

Cytokine Mediators of Inflammation: Interleukines

What are features of interleukins?

- Interleukins are made in helper CD4+ T lymphocytes, but also in monocytes, macrophages, and endothelial cells.
- Interleukins promote development and differentiation of T and B lymphocytes and hematopoietic cells
- In humans, they are more than 50 different interleukins identified; these are generally subdivided into 15 recognized types. Each has a different function. Their actions go well beyond the original identification of immune regulation.
- They appear to play a role in many diseases, such as rheumatoid arthritis.

What are effects of interleukins on inflammation?

- Interleukins are a family of cytokines with a wide range of functions, including both pro- and anti-inflammatory effects.
- They interact with T-cells and B-cells, stimulating development and differentiation. Also involved with haematopoetic cells.
- They are produced by a wide range of cells, but most notably by leukocytes, especially CD4+ T-helper cells and macrophages.
- The chief pro-inflammatory interleukins (1pm, 2ow, 3pm) are IL-1, IL-8, IL-12, and IL-18. Anti-inflammatory interleukins are IL-4, IL-9 IL-10, IL-11, IL-13, and IL-19.

What does IL-1 do?

- The interleukin (IL)-1 family of cytokines has 11 members, including 7 pro-inflammatory agonists and 4 exerting anti-inflammatory activities (1pm).
- IL-1s are produced primarily by macrophages, monocytes, dendritic cells, fibroblasts.
- They influence innate inflammation and acquired immunity (1pm).
- Commonly reported other actions include:
  1) Stimulate adhesion of endothelial cells.
  2) Activate hypothalamus to raise body temperature (fever).
  3) Increase pain sensation.
  4) Cause vasodilation and hypotension.

What does Interleukin-8 do?

- Interleukin-8 induces chemotaxis towards inflammatory site in neutrophils and granulocytes,; IL-8 stimulates phagocytosis on arrival.
- IL-8 is produced by all cells with toll-like receptors (TLR).
- These "sentinel" cells include macrophages, dendritic cells, natural killer cells, T and B cells, and some non-immune cells (endothelial, epithelial, fibroblasts.
- IL-8 induces histamine release and respiratory burst (release of reactive oxygen species).

What does IL-18 do?

- IL-18 (1w, 2rg, 3sd) is a member of IL-1 superfamily.
- IL-18 is chiefly produced by macrophages and has the following actions:
  1) Stimulates release of, and is upregulated by, interferon-gamma.
  2) Directly promotes severe inflammatory reaction.
  3) Implicated in Hashimoto's thyroiditis (a common auto-immune disease[1w]).

What does IL-12 do?

IL-12 does the following:

1. Responds with increased IL-12 production by antigenic stimulation of macrophages, dendritic cells, neutrophils, and B-lymphoblastoid cells.
2. Stimulates naïve T-cell differentiation into type I T Helper cells (Th1)
3. Stimulates production of interferon-gamma and TNF-alpha from T cells and natural killer cells.
4. Enhances activity of natural killer cells and cytotoxic (CD8+) T-cells.
5. Shapes autoimmune reactions.

What are inflammasomes?

- **Inflammasomes are a multiprotein intracellular complex signalling cascade that (a) detects pathogenic microorganisms or sterile stressors, and (b) that stimulate production of highly pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18.**

Cytokine Mediators of Inflammation: Lymphokines

What are lymphokines?

Lymphokines are cytokines produced by T lymphocytes. Examples include:

- colony-stimulating factors (CSFs), including GM-CSF
- interferons (IFNs) - IFNγ
- interleukins IL-1 to IL-8, IL-10, IL-13
- macrophage inflammatory protein-1 beta (MIP-1β)
- neuroleukin (lymphokine product of lectin-stimulated T cells)[s]
- osteoclast-activating factor
- platelet-derived growth factor (PDGF)
- transforming growth factor beta (TGFβ)
- tumour necrosis factor-alpha (cachectin) (TNFα)
- tumour necrosis factor-beta (TNFβ, lymphotoxin α, LT)

What are the features of tumour necrosis factor alpha?

- Tumour necrosis factor (TNF, tumour necrosis factor alpha or cachexin) is a cytokine contributing to systemic inflammation (and the acute phase reaction).
- It is produced by activated macrophages (primarily); can also be produced by neutrophils, eosinophils, mast cells, some neurons, natural killer cells, T-helper (CD4+) lymphocytes, and other cells.
- TNF promotes inflammation and apoptosis, and inhibits tumorigenesis and viral replication.
- It is released in large quantities in response to lipopolysaccharides (and other bacterial products) and interleukin-1

What are the actions of TNF-α?

1. Increased local concentration promotes all the classic signs of inflammation – rubor, tumour, calor, dolor, functio laesa.
2. Systemically, promotes fever, loss of appetite, release of corticotrophin releasing hormone.
3. Neutrophil chemoattractant and promoter of endothelial adhesion molecules (aiding neutrophil migration)
4. Stimulates macrophage phagocytosis, prostaglandin E2 and IL-1 production.
5. Stimulates systemic and hepatic acute phase response.
6. High concentrations → shock symptoms; prolonged low concentrations → cachexia (1).

What is the clinical use of anti-TNF drugs?

Anti-TNF drugs such as infliximab and adalimumab, among many others, are often used to treat clinical problems of autoimmune diseases, e.g. inflammatory bowel disease, lupus erythematosus, asthma.

Mediator of Inflammation:
Platelet Activating Factor

What are the actions of platelet-activating factor in inflammation?

Platelet-activating factor (1pm)is a potent phospholipid activator and mediator of many leukocyte functions. Its many actions include:

1. **Platelet aggregation and degranulation**
2. **Promoting inflammation**
3. **Causing anaphylaxis**
4. **Changes in vascular permeability**
5. **Causing oxidative burst**
6. **Chemotaxis of leukocytes**
7. **Augmentation of arachidonic acid metabolism in phagocytes.**

Mediators of Inflammation: Eicosanoids

What are eicosanoids?

- Eicosanoids are metabolites of arachidonic acid.
- They can be both pro- and anti-inflammatory.
- They are produced by a variety of tissues and cells.

What are several major actions of eicosanoids?

1. Promoting inhibiting inflammation, allergy, and other immune responses.
2. Regulating abortion of pregnancy and normal childbirth
3. Regulating perception of pain
4. Regulating cell growth
5. Regulating blood pressure
6. Modulating the regional flow of blood to tissues.

What is cyclooxygenase?

- Cyclooxygenases (COX1 an d COX2) are the key enzymes in the pathway to prostanoids from arachidonic acid.
- Prostanoids are a subclass of eicosanoids including

1. Prostaglandins (mediators of inflammatory and anaphylactic reactions)
2. Thromboxanes (mediators of and platelet aggregation vasoconstriction)
3. Prostacyclins (active in the resolution phase of inflammation.)

What are key prostaglandins?

Prostaglandin E2 (PGE2) - the most abundant prostaglandin

Other prostaglandins have been discovered. Among these are

- Prostaglandin I2 (PGI2)
- Prostaglandin D2 (PGD2)
- Prostaglandin F2alpha (PGF2alpha)

What are example actions of important prostaglandins?

- **PGI2 (vasodilation, inhibits platelet aggregation, bronchodilation)**
- **PGD2 (produced by mast cells, promotes allergic diseases such as asthma)**
- **PGE2: EP1 (bronchoconstriction, GI smooth muscle contraction)**

  EP2 (bronchodilation, GI smooth muscle relaxation, vasodilation)

  EP3 (↓ gastric acid, ↑ gastric mucus, uterus contraction, GI smooth muscle contraction, lipolysis inhibition, ↑ autonomic neurotransmitters, ↑ platelet response to agonists, atherothrombosis)

What are the major thromboxanes?

- Thromboxane A2 (1i)

  Actions are:

  a) Vasoconstriction

  b) Platelet aggregation → thrombosis

- Thromboxane B2

  a) Inactive metabolite of thromboxane A2

What are the main products of the cyclo-oxygenase pathway

- **Cyclooxygenase pathways (COX 1 and 2) produce:**

  - Prostaglandins – contribute to fever, general pain perception, pain sensitization, and the inflammatory process as a whole.

  - Prostacyclins – vasodilators, inhibit platelet activation

  - Thromboxane – stimulates clotting; vasoconstrictor; TXA_2 is suspected to be a proinflammatory mediator.

What are the actions of prostacyclin?

- **Inhibits platelet activation**
  a. **Prevents platelet aggregation and thrombosis**
  b. **Prevent vasoconstriction (promotes vasodilation)**
- **Other actions**
  a. **Anti-proliferative**
  b. **Anti-inflammatory**
  c. **Anti-mitotic**

What are lipoxygenases?

- Lipoxygenases are a family of non-heme enzymes that catalyze polyunsaturated fatty acids into lipids that act as autocrine-signaling and paracrine-signaling molecules.
- The three major types of lipoxygenases are:
  a. 5-lipoxygenase
  b. 12-lipoxygenase
  c. 15-lipoxygenase

What are the main products of the 5-lipogenase pathway?

- The 5-lipogenase pathway (1pm) leads to potent proinflammatory leukotrienes (LTs).
- Leukotrienes are a family of eicosanoid inflammatory mediators produced in leukocytes by the action of 5-lipoxygenase on arachidonic acid or eicosapentaenoic acid.
- One of the actions of leukotrienes is to cause contractions of bronchiolar smooth muscles. They also cause inflammation of asthma and allergic rhinitis.

What are the main products of the 12-lipoxygenase pathway?

- **The main products of 12-lipoxygenase are:**
  - **12(S)-HPETE and 12(S)-HETE (12-Hydroxyeicosatetraenoic acid): these are most studied in platelets, but have activities in other tissues**
  - **Lipoxins: act to resolve inflammatory responses**

What are products of 15-lipoxygenase action?

- 15-lipogenase action on arachidonic acid (omega-6) or omega-3 fatty acids (eicosapentaenoic acid [EPA] and/or docosahexaenoic acid [DHA]) yields a variety of lipoxin, resolvin, and protectin metabolites, which appear to inhibit, limit, or resolve various inflammatory diseases.

What do the components of the arachidonic acid pathway do?

Prostacyclin (PGI_2) - Stimulates vasodilation, inhibits platelet aggregation

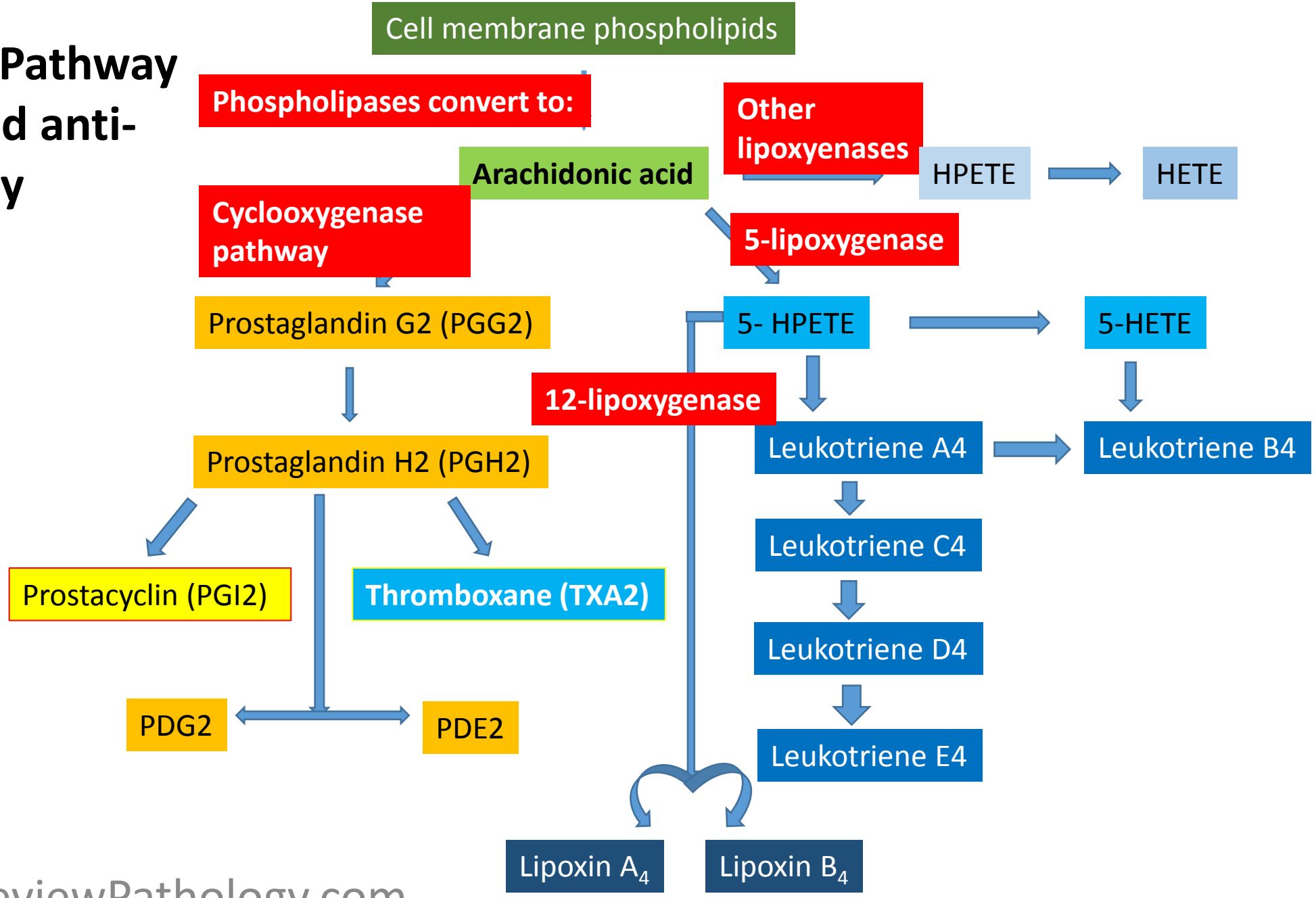
Thromboxane A_2 - Stimulates vasoconstriction, promotes platelet aggregation

Leukotrienes C_4, D_4, E_4 - Promote vascular permeability, vasoconstrction, bronchospasm, mucus secretion

PDG_2 and PDE_2 - Promote vasodilation, vascular permeability, pain

Lipoxin A_4 and B_4 - Inhibit adhesion and chemotaxis of neutrophils

Arachidonic Pathway
Both pro- and anti-inflammatory



What steps of the arachidonic acid pathway are commonly inhibited clinically?

Phospholipase conversion of cell membrane phospholipids to arachidonic acid – inhibited by steroids (used for various purposes).

Cyclooxygenase (COX_1 and COX_2) – inhibited by NSAIDs used for relieving pain and inflammatory symptoms.

Lipooxygenase (LOX) – inhibited by zileuton (used for asthma maintenance).

Leukotrienes C_4 and D_4 – inhibited by montelukast and zafirlukast (used for asthma maintenance and seasonal allergies).

How do NSAIDs work?

- **NSAIDs (non-steroidal anti-inflammatory drugs) largely work by targeting arachidonic acid metabolism.**
- **The main relevant pathways are COX-1 and COX-2.**
- **Blocking these pathways diminishes prostaglandin synthesis.**
- **Hence, NSAIDs work as analgesics, lower fever, and help prevent excessive swelling.**

Mediator of Inflammation: Lysosome Granules

What role do lysosome granules play in inflammation?

- Lysosome granules contain lysosomal enzymes.
- Lysosomal enzymes are (a) made in endoplasmic reticulum, (b) are transported to the Golgi apparatus and (c) are tagged for lysosomes by a mannose-6-phosphate label.
- Granules are produced by granulocytes (neutrophils, basophils, eosinophils, mast cells)
- These cells release lysosomal enzymes, which lyse, or break down, materials, including pathogens and cellular debris.

Mediators of Inflammation: Reactive Oxygen Species

What are the reactive oxygen species?

- Superoxide
- Peroxides
- Hydroxyl radicals
- Singlet oxygen
- Nitric oxide

What is the role of reactive oxygen species in inflammation and tissue injury?

1) Damage microbial DNA.

2) Lipid peroxidation, oxidation of protein amino acids, deactivation of specific enzymes by oxidating co-factors – all interfere with cellular function.

3) Activate interferon regulatory factors and nuclear factor kappa-B to create an anti-viral state.

4) Signaling factors upregulating cytokine production and autophagy, affecting granuloma production.

5) Involved in activation and apoptosis of T-cells.

What is the role of Nitric Oxide in inflammation?

- Produced by macrophages, some neurons, and endothelial cells.
- Promotes vasodilation and vascular permeability.
- Reduces platelet aggregation.
- Assists in leukocyte recruitment.
- Regulates function, proliferation, and apoptosis of a wide range of both lymphocytes and leukocytes.
- High concentrations are directly anti-bacterial.

Mediators of Inflammation
Specific Cell Types

What is the role of mast cells in inflammation?

- Mast cells – store heparin and histamine in pre-formed granules, ready to degranulate on stimulus.
- The effect is arteriolar dilation, increased blood flow, and vasopermeability.

Besides histamine, what else do mast cells produce, and to what effect?

- As well as histamine and heparin, mast cells produce:
- Prostaglandins…cause vasodilation, fever, pain
- Leukotrienes…increase vascular permeability; chemotaxic action
- Tryptase (a protease)…involved with vascular tone; frequent lab marker for mast cell activation.

What is the role of basophils in inflammation?

1) Basophils release histamine, heparin (slows blood clotting), and proteolytic enzymes (elastase, lysophospholipase).

2) They also secrete leukotrienes and cytokines, especially IL-4, major mediators of allergic reactions.

What is the role of T-cells in inflammation? (see details in the section on the immune system)

- T-cells are activated and differentiated by antigens on *antigen-presenting cells* (dendritic cells, macrophages); co-stimulated by antigen-presenting cells.
- Also they are induced to differentiate by cytokines and transcription factors of the inflammatory process (1pm).
- Differentiated T-cells in turn produce their own inflammatory cytokines. T_H1 cells produce interferon-γ, a cytokine critical for innate and adaptive immunity against viral, some bacterial and protozoal infections..

How do antigen-presenting cells affect inflammation?

- Antigen-presenting cells mediate the cellular immune inflammatory response by processing and presenting antigens for recognition mainly by T lymphocytes.
- They do this by breaking down proteins into smaller peptides, which are presented in association with type II MHC (major histocompatibility complex) to activate the T-cells (1, 2)
- T-cells in turn influence inflammation via Th1 cell-secretion of interferon-γ(IFNy), interleukin (IL)-2 and tumor necrosis factors (TNF), or Th2 cell-secretion of IL-4, IL-5, and IL-13.

What are the types of antigen-presenting cell?

- "Professional" antigen-presenting cells – express exogenous peptides via MHC II and use co-stimulatory signals to activate T-cells (1i, 2i, 3i, 4i)
- "Non-professional" antigen-presenting cells – display endogenous peptides via MHC I to provide a target for cytotoxic T-cells (1i, 2i, 3i).

What are the professional antigen presenting cells?

- **Professional APCs include:**
  - **Macrophages**
  - **Dendritic cells**
  - **B-cells**

All of these present antigens to and activate helper T-cells.

T-cells can only be activated by antigens that have been processed and presented. They do not respond to free antigens.

What are the non-professional antigen presenting cells?

- Non-professional antigen presenting cells include every nucleated cell that is capable of presenting endogenous peptides on the MHC1 molecule (targeting for CD8+/"cytotoxic" T-cells).
- Non-professional APCs include a variety of other types of cells (e.g., fibroblasts and cells of epithelial origin)

How do cytotoxic T lymphocytes affect inflammation?

- Antigen-presenting cells present the offending peptide to CD8+ T-cells.
- This stimulates differentiation into cytotoxic T lymphocytes (CTLs).
- Upon identifying a target, CTLs release perforin (1i), which opens the target membrane, and granzymes (1i), which enter the target cell to activate the apoptotic pathway by means of cleaving caspases.

Mediators of Inflammation: Immunoglobulins

What is the role of immunoglobulins in inflammation? (see section on the immune system)

- Immunoglobulins are plasma proteins also known as antibodies.
- Chiefly secreted by differentiated B-cells ("plasma cells")
- Can neutralize pathogens
- Can agglutinate foreign cells (for phagocytosis)
- Can precipitate serum-soluble antigens (for phagocytosis)
- Can activate the complement cascade (described in more detail in section on Immunity).

What are major actions of the different classes of immunoglobulin?

- **IgA – found in mucosal secretions (and breast milk); an early defense against orally ingested or inhaled pathogens.**
- **IgD – primarily an antigen receptor for naïve B-cells.**
- **IgE – binds to allergens, triggers mast cell degranulation; also the primary defense against parasitic invasions. An excess may lead to atopy (triad of hay fever, asthma, and eczema).**
- **IgG – main source of antibody-based immunity. Only immunoglobulin that can cross the placental barrier.**
- **IgM – secreted as pentamer, bound to B-cell surface as monomer. First defense against pathogens until IgG levels rise high enough.**

What role does the macrophage play in inflammation?

Macrophages (1pm) affect the inflammatory process in the following ways

1. Antigen presentation
2. Phagocytosis
3. Immunomodulation

    a. Production of cytokines (e.g., interferon gamma, granulocyte-monocyte colony stimulating factor, and tumor necrosis factor alpha)

    b. Production of growth factors

4. Tissue repair

How do eosinophils contribute to inflammation?

- Eosinophils are best known as mediators of allergic responses (hay fever, food allergies, atopic dermatitis, anaphylaxis and asthma). Upon activation, they release a host of pro-inflammatory factors such as:
  - —Reactive oxygen species
  - —Eicosanoids (leukotrienes and prostaglandins)
  - —Enzymes, such as elastase
  - —Growth factors (e.g, TGF beta, VEGF, and platelet derived growth factor)
  - —Cytokines (pro-inflammatory interleukins and TNF alpha)

Mediators of Inflammation: The Complement Cascade

What is the complement system?

- The complement system consists of blood proteins that when activated
  - stimulate phagocytes to clear foreign and damaged material
  - Promote inflammation to attract additional phagocytes
  - Activate of the cell-killing membrane attack complex.

The complement system is discussed in more detail in the section on the immune system

What are specific functions of complement protein fragments?

Complement protein fragments are modified products of parent complement factor. Several have unique actions.

- C3a – Also a moderate promoter of mast cell and eosinophil chemotaxis. Regulates TNF-alpha and IL-6 production in B-cells; G-protein receptor signaling (C3aR) is essential for Th1 generation and regulates expression of anti-inflammatory IL-10.
- C4a: anaphylatoxin. Also stimulates neutrophil production of pro-inflammatory cytokines
- C5a: powerful anaphylatoxin and inflammatory mediator. Significantly stronger chemotactic agent than C3a. Also stimulates phagocytosis by monocytes and neutrophils.
- - Potent pro-inflammatory effects implicate C5a strongly in complement-related inflammatory conditions: e.g., sepsis (key mediator of neutrophil dysfunction), systemic lupus erythematosus, inflammatory bowel disease.

What other cascades are involved in inflammation?

- Coagulation cascade (1pm, 2i)
- Kinin-kallikrein cascade (1a-pm, 1b-w, 1c-i, 2i, 3i)
- Fibrinolytic cascade (1w)

Mediators of Inflammation: Kinin-Kalleikrein System

What are the actions of the Kinin-Kalleikrein cascade?

- Factor XII of the clotting cascade is activated (becomes XIIa)
- Factor XIIa cleaves prekallikrein (1w) to produce kallikrein (1w, 2i) .
- Kallikrein cleaves kininogens (1w) (inactive pro-proteins) to produce bradykinin (1w) and kallidin (a similar protein).

What is bradykinin?

- Bradykinin is an inflammatory mediator.
- It is a peptide that causes blood vessels to dilate (causing blood pressure to fall)
- It acts to dilate blood vessels by release of prostacyclin, nitric oxide, and other factors.

What are the actions of bradykinin?

Potent vasodilator.

Reduces blood pressure (vasodilation + natriuresis).

Contracts of non-vascular smooth muscle (bronchi, stomach, bowel).

Thought to be strongly involved with pain mechanism.

Note: the dry cough which is the most common side effect of angiotensin-converting enzyme (ACE)-inhibitors is probably a result of increased bradykinin causing bronchoconstriction.

Mediators of Inflammation: Coagulation and Fibrinolysis*

*** For details of these pathways, see section on haemodynamics**

How are coagulation and fibrinolysis active in acute inflammation?

- Inflammation stimulates clotting and impairs both anti-coagulant mechanisms and fibrinolysis.
- Anti-coagulant cascades are anti-inflammatory; downregulation of anti-coagulation amplifies inflammation.
- Extreme fibrinolysis are characteristic of severe sepsis, thrombosis, and disseminated intravascular coagulation (DIC).

Outcomes of Acute Inflammation

What are the outcomes of acute inflammation?

1) Resolution
2) Fibrosis/scarring
3) Chronic inflammation (including granulomatous inflammation)

Resolution of Inflammation (see section on Tissue Repair)

How does inflammation resolve?

- **Complete resolution – usually where tissue damage was minimal, insult was short-lived, and tissue could replace all lost cells. Return to a normal functional (and histological) state.**
- **Resolution of inflammation involves:**

1. **Chemical mediators decay or are broken down by enzymes.**
2. **Vascular condition returns to normal.**
3. **Leukocytes produce anti-inflammatory mediators.**
4. **Lymphatic drainage and macrophage activity clear excess fluid and debris.**

Chronic Inflammation

What is chronic inflammation?

- Chronic inflammation is a long-term inflammatory reaction.
- In active inflammation, tissue damage occurs simultaneously with healing.
- Characterized histologically by mononuclear cell (macrophages, lymphocytes, plasma cells) infiltration, ongoing tissue destruction, angiogenesis, and fibrosis.
- Cachexia, or wasting, may be seen due to TNF-α acting as an appetite suppressant, increasing fat usage, and promoting skeletal muscle wasting. TNF-α is not the only cytokine implicated in this process, but it is the most characteristic.

What are the causes of chronic inflammation?

1) Persistent infections – viral, mycobacterial, syphilis, some fungi.

- Result in a type 4 hypersensitivity reaction (delayed-type hypersensitivity).

2) Auto-immune inflammatory conditions – inappropriate auto-antigens interpret host tissue as a threat, prolonging inflammatory response indefinitely.

3) Ongoing exposure to toxins or irritants (e.g., silicosis)

What are other names for macrophages?

- **Kupffer cells (liver)**
- **Sinus histiocytes (spleen and left nodes)**
- **Microglia (central nervous system)**
- **Reticuloendothelial system (old name for systemic macrophages)**

What are M1 macrophages?

- M1 macrophages (1pm, 2pm) are typically defined those activated mainly by LPS and TNF gamma*.
- Their main action is to kill infectious bacteria
- They produce nitric oxide and reactive oxygen species
- They upregulate lysosomal enzymes
- They secrete cytokines

* Recent investigators (1pm, 2pm) suggest that the M1/M2 classification is an oversimplification.

What are M2 macrophages?

- M2 macrophages are those that function primarily in tissue repair by promotion of angiogenesis and scar tissue (fibroblast and collagen)*.
- They are stimulated by cytokines (e.g., IL-4 and IL-13)

* Recent investigators (1pm, 2pm) suggest that the M1/M2 classification is an oversimplification.

What kind of inflammation is promoted by lymphocytes?

- **Chronic inflammation**
- **Examples:**
  - **Granulomatous inflammation (e.g., tuberculosis)**
  - **Inflammation of autoimmune and hypersensitivity diseases**

What are CD4+ T (T-helper) lymphocytes?

- CD4+ T (T-helper) lymphocytes activate and modify adaptive responses.
- They recognize peptides on MHC class II molecules found on antigen presenting cells (1i, 2rg).
- There are several subsets of T-helper cells (1pm, 2i, 3rg)
  - The subset is recruited depends upon the cytokine milieu.

What are MHC class II molecules?

- **MHC class II molecules (see interesting illustrations) are a class of major histocompatibility complex (MHC) molecules (found on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B lymphocytes.**
- **Their main function is to present antigens from exogenous sources, to CD4(+) T-lymphocytes.**
- **MHC class II molecules are necessary for the antigen-specific immune response.**

What are T_H 1 lymphocytes?

- T_H 1 lymphocytes (1w) are cells stimulated by the cytokine IFN-γ to activate macrophages.

What are T_H2 lymphocytes?

- **T_H2 lymphocytes are cells that secrete IL-4, IL-5, and IL-13 and recruit and activate eosinophils and macrophages.**

What are T_{H17} lymphocytes?

- **T_{H17} lymphocytes are cells that secrete IL-17 among other cytokines to recruit neutrophils.**

What are macrophage–lymphocyte interactions in chronic inflammation?

- **Review available illustrations available on Google and Bing images (1i, 2i, 3i, 4i)**

What diseases are characterized by chronic inflammation?

- **See linked internet images for diseases associated with chronic inflammation**

Granulomatous Inflammation

What is granulomatous inflammation?

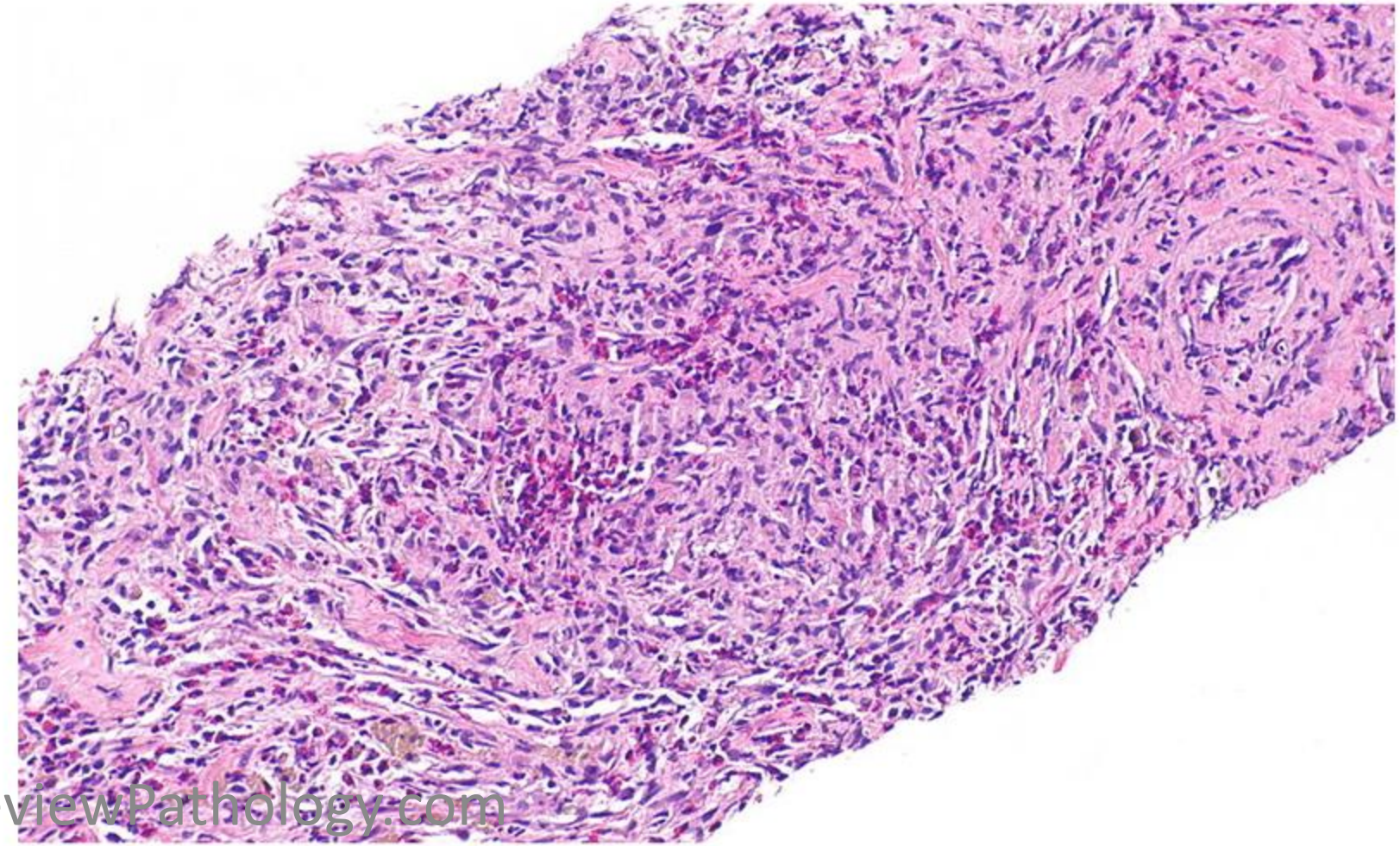
- **Granulomatous inflammation is a specific type of reaction to chronic delayed-type hypersensitivity.**
- **Macrophages infiltrate and are activated, becoming epithelioid cells (large, flat, eosinophilic) and often fusing to form multi-nucleated giant cells.**
- **A granuloma is an aggregate of epithelioid cells surrounded by a ring of lymphocytes; it may also be enclosed by fibroblasts and connective tissue.**

What causes granulomatous inflammation?

- The most notorious cause of granulomas is tuberculosis.
- Other causes include: sarcoidosis, Crohn's disease, histoplasmosis, leprosy, and cat scratch disease.
- Necrosis in the centre of a granuloma often indicates an infective cause.
- Caseous necrosis (appears cheese-like on gross inspection) is strongly associated with, though not pathognomic for, tuberculosis.

Granulation tissue containing an eosinophilic granuloma with Langerhans cell

histiocytosis(https://commons.wikimedia.org/wiki/File:Pulmonary_Langerhans_cell_histiocytosis_--_intermed_mag.jpg



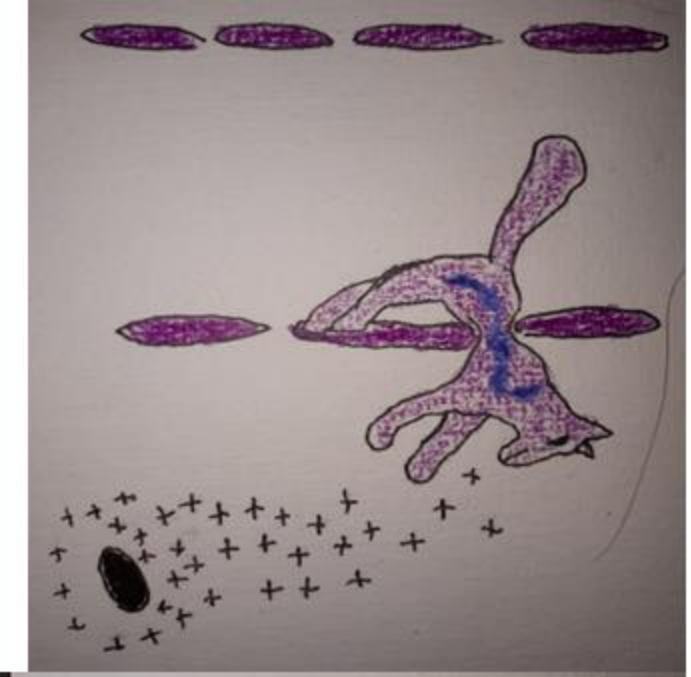
THE ADVENTURES OF NEUTROPHIL DOG: A Dog's Eye View of Diapedesis, Chemotaxis, and Phagocytosis!

Margination:
“All these fast-moving little red terriers are pushing me to the side!”

Initial adhesion:
“Oh no, my paws are getting sticky!”

Rolling – “My paws were sticky, maybe I should roll!”

Adhesion, recognition of increased vascular permeability, and detection of chemokines – “Argh, my paws are stuck…but wait, is that a hole? And do I smell something interesting?”



Dogapedesis – “I do smell something interesting! And I can sqeeze through this hole and chase the scent gradient until I can…

PHAGOCYTOSE!

Recruitment – “There’s lots of food here. I should call in my whole pack!”