# INFLAMMATION

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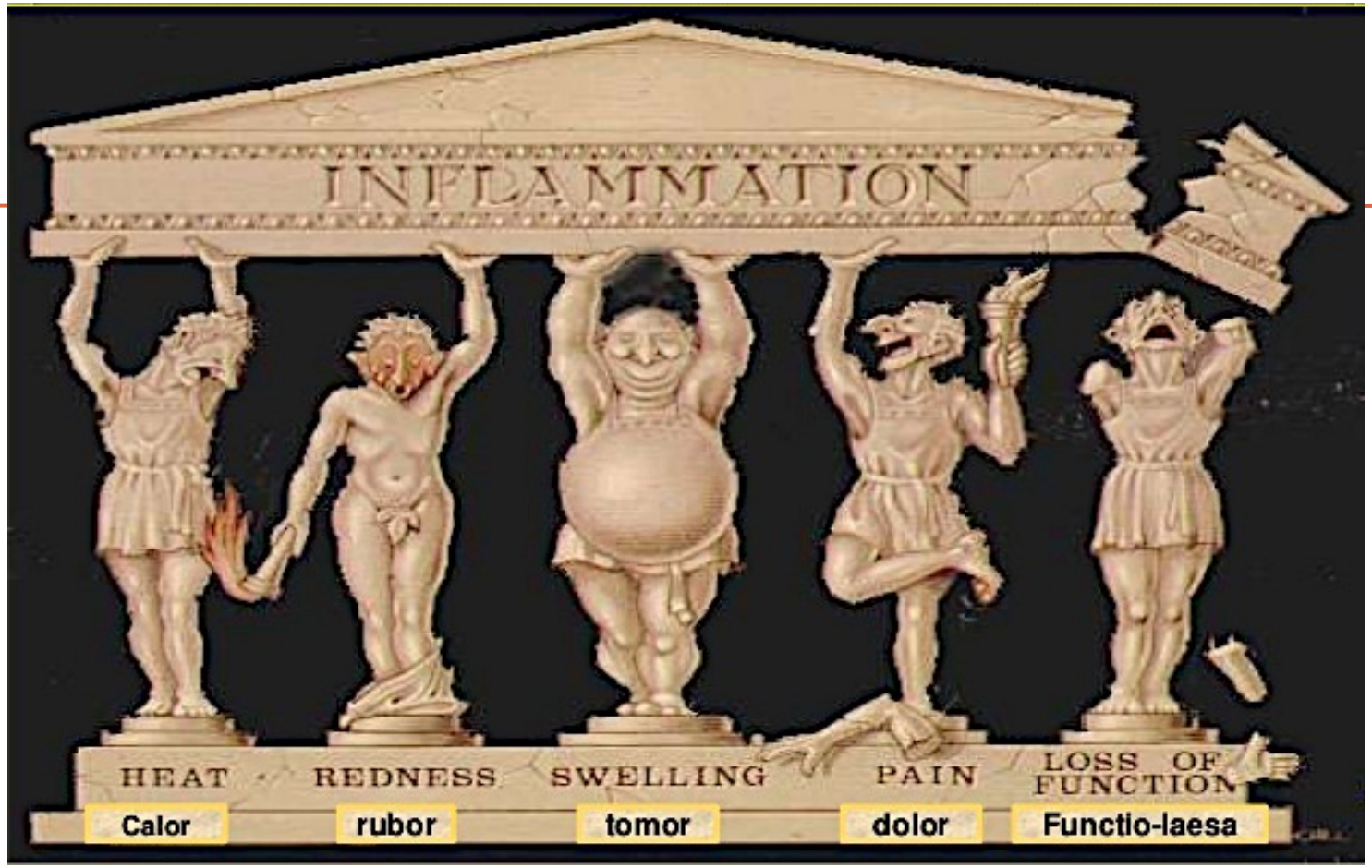
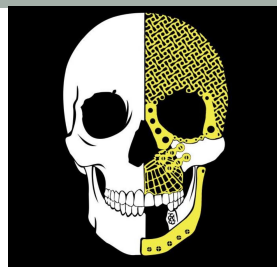
GEN PATH OMFS SOCIETY D2 ELEVATOR

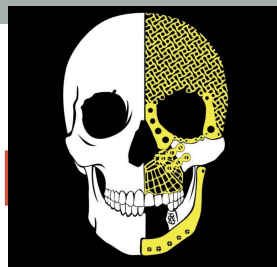


# Inflammation



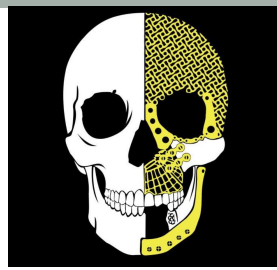
- Acute inflammation
  - Innate Response
    - **HALLMARK**: EDEMA and NEUTROPHILS (PMNs, peak at 24 hrs)
    - Early edema ➔ transudate (ultra-filtrate of blood)
    - Late edema ➔ exudate (cells/enzymes)
- Chronic inflammation
  - Adaptive response
  - **HALLMARK**: LYMPHOCYTES (T-CELLS, B-CELLS), PLASMA CELLS, MACROPHAGES

# Clinical signs of Acute Inflammation



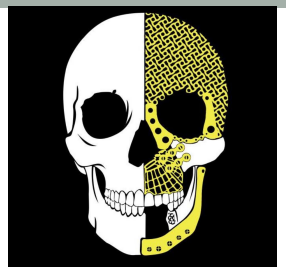
1. Vasoconstriction
   - Blanching
2. Vasoldilation
   - Redness
3. Edema
   - Swelling

# What triggers inflammation

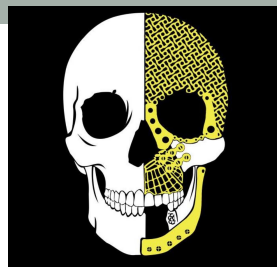


- Infection
- Tissue necrosis
- Foreign bodies
- Immune reaction
- Trauma

# Inflammation in a nutshell– First step of wound healing



1. Recognize foreign body or damage
2. Activate immediate response (NFkB)
    1. Vasodilation to allow for a response
    2. Cells to respond
3. Cells respond
4. Outcome



Tissue injury or infection

Production of inflammatory mediators

**Vaso-active mediators**
- Histomine
- Serotonin
- Bradykinin
- Leukotrienes/prostaglandins
- Platelet activating factor
- Nitric oxide
- Cytokines

**Chemotactic factors**
- C5a
- $LTB_4$
- fMLP
- chemokines

Rubor (red) &
Calor (warmth)

**Vasodilation**
**Increased permeability**

**Inflammatory cell recruitment and activation**

Edema

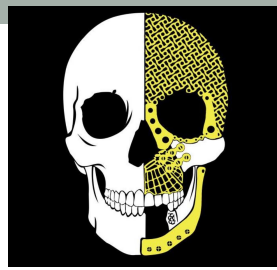
Swelling (tumor)

Acute inflammation

Chronic inflammation

**Sources of VAM**

| | | | |
|---|---|---|---|
| **From cells in the affected area** | Mast cell & basophil | → | Histamine |
| | Platelets | → | Serotonin |
| | MΦ & inflammatory cells | → | PAF, PG, LKT, Cytokines |
| | Endothelium | → | PAF, PG, NO |
| **From circulation** | Hageman factor activation | Clotting system | Fibrin split products |
| | | Kallikren-kinin system | Kinins (bradykinins) |
| | Complement system activation | | C3a, C5a |

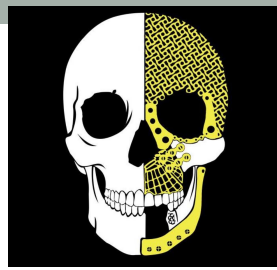


20. An asthmatic attack results in IgE mediated mast cell degranulation followed by edema, excessive mucous secretion and spastic smooth muscle airway contraction. The vasoactive mediator primarily responsible is:

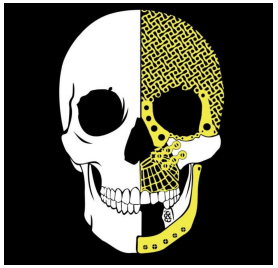
A. prostaglandin $PGE_2$
B. $TxA_2$
C. $LTB_4$
D. histamine
E. serotonin

D

# Inflammation



1. TLRs on dendritic cells & macrophages recognize PAMP/DAMPs
   - Ex: CD14 is a TLR-4 on macrophages and can recognize LPS (PAMP specific to Gram – bacteria only)
   - Ex: Nod-like receptors recognize PAMPs and DAMPs convert into ➔ inflammasomes ➔ induce apoptosis OR release IL-1 and IL-8
2. TLR activation results in up-regulation of NFkB pathway
3. Immune mediators released ➔IL-1, IL-6, TNFa, chemokines (IL-8)
   - Ex: arachidonic acid released by Phospholipase $A_2$ enters one of two paths:
     - Cyclooxygenase (COX) ➔ Prostaglandins (PG) ➔ vasodilation (arterioles) and increased vascular permeability
     - 5-lipooxygenase (LOX) ➔ Leukotrienes (LT) ➔ attract and activates PMNs
4. Outcomes
   - Resolution
   - Chronic inflammation
   - Fibrosis



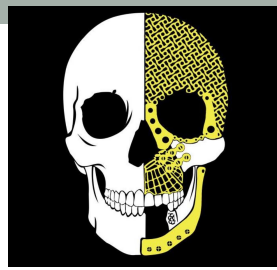
43. Why are TLRs critical to the subsequent immune response?

A. They activate specific transcription factors that determine the subsequent immune response.

B. They prevent the spread of the pathogen into the circulation.

C. They induce phagocytosis to control bacterial replication.

D. They are the only part of the immune system that can recognize viruses.



41. Toll-Like-Receptors (TLRs) can recognize bacterial products because:

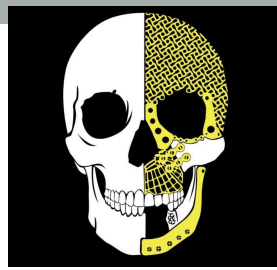
A. Receptors recognize ligands

B. Random binding permits TLR bearing cells to phagocytose bacteria.

C. The bacteria are coated with complement proteins which the TLRs recognize.

D. Bacteria have unique biochemical properties

D

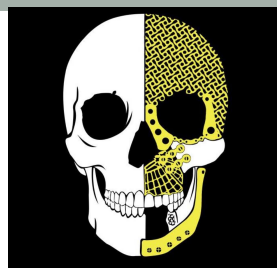


31. After binding endotoxin by Toll-like receptor-4, the transcription of tumor necrosis factor α (TNF-α), interleukin 1 and 6 (IL-1 and IL-6), in macrophages is mediated by:

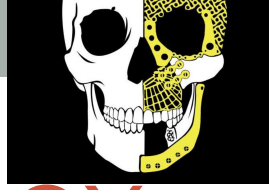
A. the cyclooxygenase pathway
B. the lipoxygenase pathway
C. AP-1 expression
D. NFκB
E. interferon γ

D

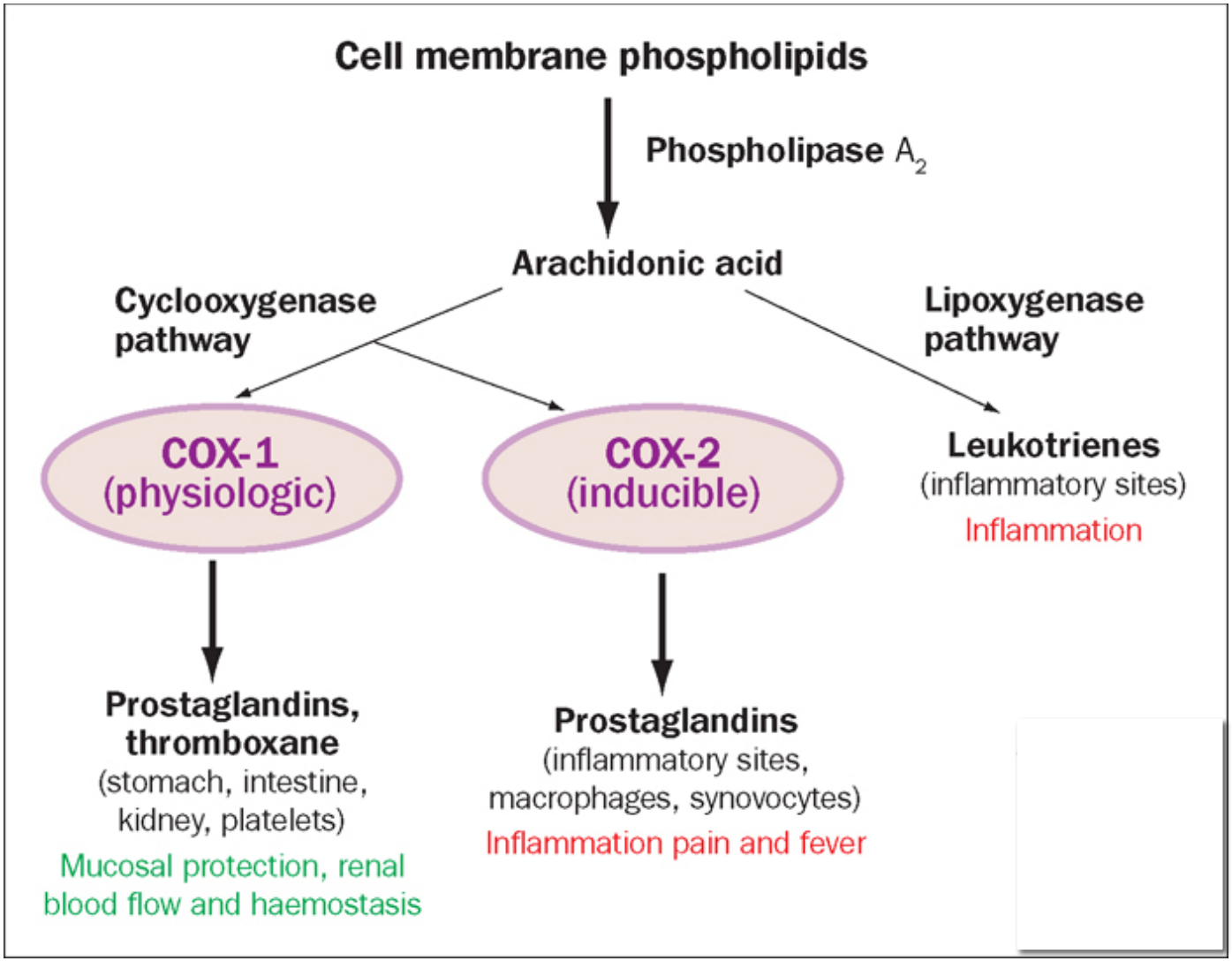
# What happened so far?



1. Recognized foreign body/injury
2. Activated NFkB
3. Activation of co-stimulatory molecules
4. Activated $PLA_2$
5. What happens next?
   - We need to vasodilate in order to bring in an army to fight infection



# Arachidonic Acid Pathways – COX & LOX



# Cell-derived mediators: arachidonic acid pharmacologic inhibition

Phosphatidylcholine

*P-lipase* $A_2$

corticosteroids

Arachidonic acid

*P-lipase C*

Phosphatidyl inositol–$P_2$

Cycloxygenase pathway

Aspirin, COX-2 inhibitors, NSAIDS

Prostaglandins
- $PGE_2$ Fever + pain
- $PGI_2$ inhibitor

Thromboxanes
- $TxA_2$ Platelets + SM contraction

Lipoxygenase pathway

Zileutin

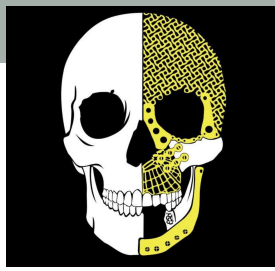
HPETES → HETES
Vaso-active mediators

Leukotriene $A_4$ → $LTB_4$
PMN recruitment factor

$LTC_4$ → $LTD_4$ → $LTE_4$ = SRS-A
SM contraction
Permeability

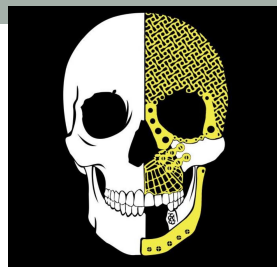
# Arachidonic Acid Pathways – COX & LOX

| COX Pathway | |
|---|---|
| *$PGE_2$* | - Expressed early in inflammation<br>- PMN & Monocyte recruitment<br>- ***Edema*** |
| *$PGI_2$* | - Expressed during resolution by endothelium<br>- Antagonist of $PGE_2$<br>- ***Edema*** |
| *TxA* | - Synthesized by platelets<br>- Platelet adherence<br>- ***Edema*** |
| **LOX Pathway** | |
| *HETES* | - VAM<br>- ***Edema*** |
| *$LTB_4$* | - VAM<br>- PMN recruitment<br>- ***Edema*** |
| *Slow Reacting Substance of Anaphylaxis (SRS-A)* | - Synthesized by Mast Cells, Basophils, Eosinophils<br>- SRS-A dislodge parasites from mucosal membrane by<br>1. By increasing ***edema***<br>2. By triggering smooth muscle contraction<br>3. By mucin secretion |



# Anti-inflammatory Agents

- Corticosteroids ➔ phospholipase A
  - Inhibits BOTH pathways
- Aspirin ➔ COX-1 and COX-2
  - IRREVERSIBLE INHIBITOR
  - Inhibits both tissue hemostasis and inflammation
  - Problem in platelet aggregation (bleeding)
- NSAIDs ➔ COX-1 and COX-2
  - REVERSIBLE INHIBITOR
  - Inhibits both tissue hemostasis and inflammation
- Vioxx ➔ COX-2
  - Associated with heart attacks
- Celebrex➔ COX-2
- Zileutins➔ LOX



17. A bilateral sagittal split of the mandibular ramus is planned to correct a skeletal malocclusion however the procedure may result in extensive edema that could compromise the patient's airway. Therefore a course of corticosteroids is begun the day prior to surgery. Which of the following vasoactive mediators would be inhibited by corticosteroid administration.

A. $PGE_2$

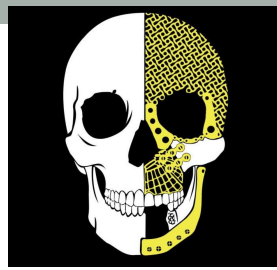
B. platelet activating factor

C. $LTB_4$

D. SRS-A

E. all of the above

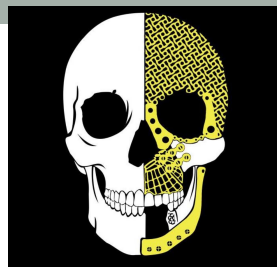
E



18. You extract several compromised teeth for a patient who states that he is not taking any medications but soon experience problems controlling post extraction bleeding. Upon questioning, he relates that he has been taking aspirin (81 mg/day) to prevent heart attacks. Difficulty in obtaining hemostasis is most likely due to aspirin's effect on:

A. $PGE_2$
B. $TxA_2$
C. $LTB_4$
D. SRS-A
E. $PGI_2$

B

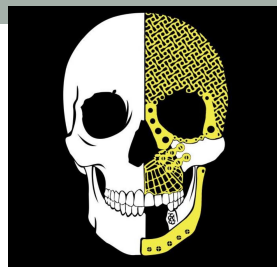


19. Prior to oral surgery you administer 600 mg of Ibuprofen for its analgesic and anti-inflammatory properties. Ibuprofen functions as a:

A. irreversible COX inhibitor
B. reversible COX inhibitor
C. non-competitive COX inhibitor
D. endorphin antagonist
E. both C and D

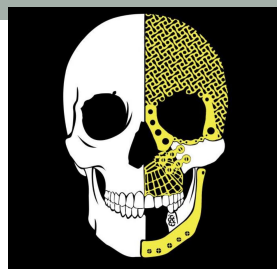
B

# Inflammatory Mediators



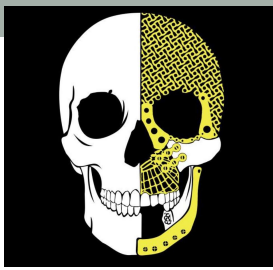
| *Mediators* | *Secretion* | *Functions* |
|---|---|---|
| Platelets<br><br>Platelet Activating Factor (PAF) is released by $PLA_2$ ➔ stimulates platelet aggregation and degranulation and primes inflammatory cells | **(Δ) Dense granules** release *serotonin, $Ca^{2+}$, and ADP* | - **Serotonin**: VAM promoting edema<br>- **$Ca^{2+}$** and **ADP**: blood coagulation |
| | **(α) Alpha granules** release cationic proteins, fibrinogen, and Platelet derived growth factor | - **Cationic proteins** neutralize the charge of endothelial cells and RBC<br>- **Fibrinogen**: the end of blood clotting pathway<br>- **PDGF**: initiation of the wound healing process (“Competence Factor”) |
| | **(λ) Lyzosomal vesicles** release acid hydrolases | |
| | **Thromboxane $A_2$** | - Platelet adhesion |

# Inflammatory Mediators



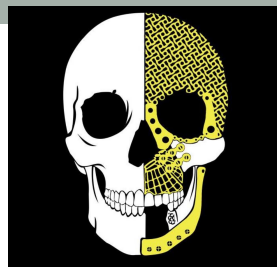
| ***Mediators*** | ***Secretion*** | ***Functions*** |
|---|---|---|
| Mast Cells & Basophils | - **Dense granules** release <u>histamine, serine proteinases and *chemokines*</u> | - IgE Receptors binding epitope→ SRS-A synthesis and release with granule contents<br>- Histamine: edema, smooth muscle contraction<br>- Chemokines: N phils & E phils |

# Inflammatory Mediators



| ***Mediators*** | ***Secretion*** | ***Functions*** |
|---|---|---|
| Endothelial Cells<br><br>(local tissue perfusion regulators) | - PGI2<br>Nitric Oxide,<br>Endothelin,<br>Procoagulation tissue factor | - **PGI2**: vasodilator & anti-aggregation factor; antagonist to PGE2<br>- **NO**: smooth muscle relaxant → arteriole dilate → edema<br>- **Endothelin**: venuole constrictor, → increase hydrostatic pressure at the capillary bed<br>- **Procoagulation tissue factor** in response to LPS, IL1, or TNFa |
| MΦ | - TNF-a, IL-1, IL-6 | - **TNF-α**: ↑ vascular permeability and expression of endothelial CAMs, other cytokines, systemic effects<br>- **IL-1**: ↑ vascular permeability and expression of endothelial CAMs, other cytokines, systemic effects<br>- **IL-6**: acute phase cytokine |

# What has happened so far?

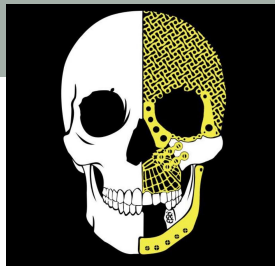


1. Recognized foreign body/injury
2. Activated NFkB
3. Activation of co-stimulatory molecules
4. Activated $PLA_2$
5. Activated Arachidonic Acid
6. Activated LOX and COX
7. Vasodilation, increased permeability
8. Released PMN attractors
9. Now how do the PMNs get there?

# Neutrophil activators & attractors

1. C5a (complement chemokine)
2. Leukotriene B4
3. IL-8
4. Bacterial products

# Targeted Cell Recruitment



25. The initial recruitment of neutrophils during an innate immune response from the vascular to extra vascular space (also called margination), occurs through the expression of specific selectins on the luminal surface of endothelial cells and____ of neutrophils:

A. Sialyl Lewis$^{x}$ carbohydrates
B. Leukocyte functioning antigen (LFA-1)
C. Intrascellular adhesion molecule -1 (ICAM-1)
D. Il-8
E. CD 28 (endothelial cell recognition carbohydrate)

A

# Targeted Cell Recruitment

1. **Margination (rolling adhesion)**
   - PMNs usually roll along the inside of blood vessels
   - Selectin on endothelial cells bind Sialyl-Lewis X Carbohydrate (SXC) on PMN to slow them down
   - IL-8 promoted slowing down

2. **Adherence**
   - Inflammatory mediators ➔Increase integrin expression on PMN (upregulated by C5a and $LTB_4$) binds strongly to I-CAM on endothelial cells (upregulated by TNFa and IL-1)
     - ICAM 1 (endothelial cell) binds LFA-1 (PMN)
     - ICAM 2 (endothelial cell) binds CR3 (PMN)

3. **Emigration (transmigration)**
   - Caused by C5a, bacterial products (PAMPs), arachodonic acid metabolites (LTB4) and chemokines IL-8 (CXCL8)
   - PMN digest BM by using MMP14
   - Holes in the membrane allow more cells to flow out of the vessels into ECM ➔exudate!!!

12. In the development of an inflammatory response, which of the following choices are arranged in the correct sequence?

A. Increased vascular permeability, vasodilitation, increased blood viscosity, WBC emigration,WBC margination

B. Vasoconstriction, vasodilation, increased vascular permeability, increased blood viscosity, WBC margination, WBC emigration.

C. Vasodilation, vasoconstriction, increased blood viscosity, increased vascular permeability, WBC margination, WBC pavementing, WBC emigration

D. Vasoconstriction, vasodilation, increased vascular permeability, increased blood viscosity, WBC emigration, WBC margination, WBC pavementing

B

15. The passage of fluid and the selective recruitment of cells of the innate immune system from the vascular to extravascular space during an inflammatory response is effected by:

A. monocytes/macrophages
B. vascular pericytes and associated smooth muscle cells
C. endothelial cells of the microvasculature
D. resident mast cells
E. both B and C

C

# Matrix metalloproteinase family

| Enzyme | MMP | source | Substrate specificity |
|---|---|---|---|
| collagenases | 1 | CT cells | Col I, II, III |
| | 8 | Inflammatory cells | Col I, II, III |
| gelatinases | 2 | CT cells | Col IV, V, VII, X, elastin and Col I, II, III after 1° cleavage with MMP 1 or 8 |
| | 9 | CT cells | same |
| stromelysins | 3 | Many cells incl tumors | All active against proteoglycan core proteins, laminin, fibronectin, elastin |
| | 10 | | |
| | 11 | | |

23. Type I collagen comprises over 90% of the protein extracellular matrix of periodontal tissues. The degradation of fibrillar type I collagen in chronic adult periodontitis is currently thought the result of:

A. generation of reactive oxygen species by neutrophils
B. collagenases released by locally invading Gram negative bacterial species such as *Porphyromonas gingivalis*
C. host expression of matrix metalloproteinase 1
D. host expression of matrix metalloproteinase 8
E. bacterial expression of matrix metalloproteinase 8

D

# What has happened so far?

1. Recognized foreign body/injury
2. Activated NFkB
3. Activation of co-stimulatory molecules
4. Activated $PLA_2$
5. Activated Arachidonic Acid
6. Activated LOX and COX
7. Vasodilation, increased permeability
8. Released PMN attractors
9. PMNs break through BM and enter ECM
10. Now what will the PMNs do?

# Mechanisms of PMN killing: phagocytosis

1. Neutrophils have receptors for: IgG, IgM, C5a, C3b, LTB4, fMLP, IL-8, TNFa. These factors are used for recognition and phagocytosis. Granules contain:

| | |
|---|---|
| **Primary granules (P E C A M)** | - elastase (MMP-2, 9), cathepsin G, phosphorylase $A_2$, myloperoxidase and acid hydrolyases |
| **Secondary granules (L L P C)** | - phosphorylase $A_2$, lysozyme, lactoferrin and collagenase (MMP-8)<br>▪ MMP8 uncovers bacteria from host ECM*** |
| **Tertiary granules ( C G)** | ○ cathepsin and gelatinase (MMP-2, 9) |

2. Oxidative killing ➔ ROS bursts the bacteria

$$O_2 \xrightarrow{NADPH\ OXIDASE} O_2^- \xrightarrow{SOD} H_2O_2 \xrightarrow{Myleoperoxidase} Hyperchlorous\ Acd$$

$$O_2 \xrightarrow{NADPH\ OXIDASE} O_2^- \xrightarrow{SOD} H_2O_2 \xrightarrow{Fe2+(ferrous)} \dot{O}H$$

3. Non-oxidative killing: lysozyme (Gram +), defensins (Gram -), lysosomal hydrolases, lactoferrin (iron chelator)

24. The generation of reactive oxygen species such as hydrogen peroxide and hyperchlorous acid is associated with which of the following neutrophil enzymes?

A. myleoperoxidase
B. lysosomal hydroxylase
C. lactoferrin
D. lysozyme
E. bactericidal/permeability increasing protein

A

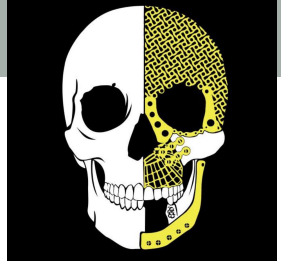
# Host tissue destruction

- Release of ROS from dead PMN cause tissue damage
- Decreased tissue perfusion
- Expression and activation of MMPs
- Inflammatory mediated bone resorption ➔ Osteoimmunology

# Inflammatory mediated bone resorption: Osteoimmunology

- RANK receptor ➔ on osteoclast
- RANK-Ligand ➔ on osteoblast
- OPG released by osteoblast ➔inhibitor of osteoclasts

- Infection ➔ endothelial cells recruit monocytes MCSF (mphages, Fblasts, Osteoblasts) + costimulatory molecules (TNFa, IL-1, IL-6) ➔ trigger monocytes to express RANK ➔ RANK binds RANK-L➔ **NFkB pathway activation** ➔ differentiate into osteoclasts ➔ **bone resportion occurs**
- OPG ➔ inhibitor released from osteoblast and fibroblast to induce osteoclast apoptosis
- Balance of OPG vs. RANKL determines formation or resorption



30. A patient who presents for implant therapy has been prescribed an inhibitor to the receptor activator of NFK-B ligand (RANKL) (Denosumab®) for the management of rheumatoid arthritis. You are a concerned the patient will have:

A. increased susceptibility to bacterial infection
B. increased susceptibility to viral infection
C. inhibited bone resorption
D. impaired soft tissue wound healing
E. both A and B

C

# Resolution of acute inflammation

- Anti-inflammatory mediators are released to down regulate selectins and signal monocytes to clean up

1. Lipoxins
2. Resolvins
3. protectins

# Chronic inflammation

- Can follow acute inflammation or occur in response to viral, parasitic infection or malignancy
- Cellular infiltrate consists of: macrophages, plasma cells, **lymphocytes** and eosinophil
- Chronic inflammation can co-exist with acute inflammation as in periodontitis

16. The inflammatory cell infiltrate in a chronic inflammatory response is:

A. primarily neutrophils
B. plasma cells
C. macrophages
D. small lymphocytes
E. B, C, and D

E

# Granulomatous inflammation

- Wall off infection and non-digestible particles
- Classic example: TB
- Characterized by epitheloid cells (activated foamy macrophages) and granulomas