INFLAMMATION: Cellular Functions
1. Vasodilation (increased blood flow) ► CALOR & RUBOR
2. Increased microvascular permeability: fluids into tissues ► TUMOR
3. Blood flow slows (stasis) and rbc concentration ► RUBOR
Sequence of Leukocytic Vascular Events

1) Margination
2) Rolling (pavementing)
3) Emigration
4) Chemotaxis
5) Phagocytosis & Intracellular killing / degradation
6) Extracellular release of leukocyte products
7) Synthesis of biochemical mediators

Leukocyte adhesion cascade
Leukocyte Events  (movie “leukocyte rolling”)
4 main groups of adhesion molecules:

1. **Selectins**
   - E-Selectin, P-Selectin
   - L-selectin

2. **Mucin-like ligands**
   - eg Sialyl-Lewis-X

3. **Integrin** receptor family
   - CD11/CD18, etc.

4. **Immunoglobulin Superfamily Adhesion Molecules (IgSAM)**
   - ICAM-1, VCAM-1, MadCAM
   - PECAM-1
## Adhesion Molecules

<table>
<thead>
<tr>
<th><strong>Endothelium</strong></th>
<th>bind to</th>
<th><strong>Leukocytes</strong></th>
<th><strong>Effect</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin &amp; E-selectin</td>
<td>↔</td>
<td>Mucin-like ligands (Sialyl-Lewis X)</td>
<td>Rolling</td>
</tr>
<tr>
<td>IgSAM’s (MadCAM, etc.)</td>
<td>↔</td>
<td>L-selectin</td>
<td>Arrest &amp; adhesion</td>
</tr>
<tr>
<td>IgSAM’s (ICAM, VCAM)</td>
<td>↔</td>
<td>Integrins (CD11/CD18, etc.)</td>
<td>Firm adhesion</td>
</tr>
<tr>
<td>IgSAM - PECAM</td>
<td>↔</td>
<td>IgSAM - PECAM</td>
<td>Emigration</td>
</tr>
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</table>
Margination and Rolling

• Slowing of blood flow → leukocytes fall out of central column.
• Tumble (rolling) along the **endothelium of venules**
• **P-Selectin**
  - activated (immediate) by histamine, thrombin, PAF
  - expressed on platelets & endothelium by redistribution to surface
• **E-Selectin**
  - activated (1-2 hours after injury) by TNF, IL-1, etc.
Rolling (pavementing)

- P- & E-selectin bind to s-Le\(^x\)
  - not a strong bond → flow of blood causes rolling
- Endothelium appears to be essentially lined by white cells
Rolling (movie - “rolling adhesion”)
Arrest & Adhesion

- after rolling, see arrest and adhesion of leukocytes to endothelium
  - initially with binding of leukocyte L-selectin to endothelial IgSAM’s (eg MadCAM)
  - then firm adhesion with integrins to IgSAM’s
    - integrins on leukocytes bind to ICAM & VCAM on endothelium
- activated by TNF-α, IL-1 + other cytokines
Bovine Leukocyte Adhesion Deficiency (BLAD)

- Holsteins
- Autosomal recessive
- Defect in $\beta_2$ Integrins
- Results
  - Neutrophils can’t get out of blood vessel
  - Chronic, recurrent respiratory and GI disease
  - Persistently high neutrophil counts (CBC) (neutrophilia)
Canine Leukocyte Adhesion Deficiency (CLAD)

- Irish Setters
- aka canine granulocytopathy syndrome
- Defect in $\beta_2$-Integrins
- Signs
  - impaired wound healing
  - delayed umbilical cord separation at birth
  - recurrent bacterial infections without pus formation
Canine Leukocyte Adhesion Deficiency (CLAD) Test

For: Irish Setters and Irish Red & White Setters

Technically known as **Canine Leukocyte Adhesion Deficiency**, this rare but devastating condition is an inherited fatal immunodeficiency disease. Pups that inherit two recessive genes for CLAD usually die early in life from multiple severe infections, even when treated with massive doses of antibiotics.

CLAD is related to the same disease in humans (LAD) and cattle (BLAD). So far, CLAD has been found only in Irish Setters. Research on the disease was carried out in England and Scandinavia, where the carrier rate is close to 12%. However, CLAD was first identified clinically in the United States.

Reliable identification of dogs that do not carry disease genes is the key to eliminating autosomal recessive diseases such as CLAD. Now OptiGen offers a new DNA-based test that provides a method to eliminate Canine Leukocyte Adhesion Deficiency, or CLAD from the Irish Setter.

As a mutation-based gene test, the OptiGen CLAD test unequivocally and specifically identifies normal dogs. Called "genetically clear," "noncarriers" or, more formally, "homozygous normals," such dogs can pass only the gene for normal leukocyte (white blood cell) function on to all their pups.

The test also identifies carriers (heterozygous dogs) with 100% accuracy. These carriers can be safely bred to "clears." Their recessive genes can only cause disease when matched with the recessive gene of another carrier. Performed early enough, this test will accurately identify.
Emigration

- manner by which leukocytes escape from the blood into the perivascular tissues.

1. Leukocytes adhered to endothelium
   - PECAM-1 on both cells

2. Insert cytoplasmic extension (pseudopodia) into endothelial gap
   - gap formation due to:

4. Whole cell follows extension

5. Collagenase excreted → breakdown BM

Histamine, etc
Emigration - location

**Postcapillary venule**
- adequate number of inter-endothelial gaps and receptors

**Time**
- with acute inflammation
  - neutrophils peak 4-6 hours
  - mononuclear cells peak 18-24 hours
- viral → LØ’s / PC’s
- allergies → Eo’s
Chemotaxis

**Definitions**

- **Chemotaxis**
  - directional migration to a chemical gradient of chemoattractant
  - receptor-mediated process

- **Chemotaxins (= Chemoattractant)**
  - are the mediators which make leukocytes travel
  - chemoattractants include some **chemotactic cytokines** (chemokines)

**Mechanism:**

- leukocytes crawl/migrate towards highest concentration of chemoattractant
- form pseudopods & drag themselves along
Emigration & Chemotaxis (movie - “extravasation”)
Leukocyte Chemoattractants

a) **Exogenous** chemoattractants (example):
   - LPS in the wall of Gram-negative bacteria
   - foreign material

b) **Endogenous** chemottractants (examples):
   - Histamine – attracts eo’s
   - Complement, particularly C5a
     - attracts neutrophils, eo’s, monocytes, basophils
   - Fibrin-degradation products
     - attracts neutrophils
   - Leukotrienes, such as LTB₄, LTC₄
     - attracts neutrophils & eo’s
   - Chemokines:
     - cytokines (esp IL-8), that promote chemotaxis
Leukocyte Migration and Activation

When leukocytes arrive at inflammatory sites:

- Produce arachidonic acid metabolites
- Degranulate and secrete lysosomal enzymes
- Activate the oxidative burst
- Secrete cytokines
  - amplify and regulate the inflammatory process
  - modulate adhesion molecules on other leukocytes
PHAGOCYTOSIS & INTRACELLULAR DEGRADATION

Purpose:
• kill & degrade bacteria (& other Ags)

Mechanism:
• recognize the enemy
• engulf it
• degrade it with enzymes & reactive $O_2$
Phagocytosis - Mechanism

Antigen (Ag) recognition and adherence

- best when coated with opsonins:
  - leukocytes receptors for Fc fragment of Ab (esp IgG)
  - leukocytes receptors for C3b
Phagocytosis - Mechanism

1. Antigen (Ag) recognition and adherence
2. Engulfment of antigen
   • membrane surrounds & fuses → phagosome
3. Phagosome + lysosome = phagolysosome
4. Degradation of microbe
   • pH drops to 4-5
   • optimal for enzymes
Intracellular Killing & Degradation

Two ways of killing the Ag (bacteria, protozoans, fungi, etc.)

1) Oxygen-dependent mechanisms
2) Oxygen-independent mechanisms
1) Oxygen-dependent mechanisms

Respiratory burst of phagocytosis

a) NADPH reaction – produces $O_2^- + H_2O_2$

b) $H_2O_2$-Myeloperoxidase-Halide System $\rightarrow$ hypochlorous acid

\[ H_2O_2 + Cl^- \rightarrow HOCl \cdot \]

c) Haber-Weiss Reaction $\rightarrow$ hydroxyl radicals

\[ O_2^- + H_2O_2 \rightarrow \text{iron} \rightarrow HO^- + OH^- + O_2 \]

d) Nitric Oxide (NO) $\rightarrow$ peroxynitrite (ONOO$^-\cdot$)
2) Oxygen-Independent Mechanisms

**Substances within leukocyte granules**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Function</th>
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<tr>
<td>Lysozyme</td>
<td>attacks bacterial cell walls (esp Gram +ves)</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>iron binding glycoprotein (sequestration)</td>
</tr>
<tr>
<td>Trypsin and chymase (mast cell granules)</td>
<td>degrade ECM</td>
</tr>
<tr>
<td>Major basic protein</td>
<td>cationic protein of eosinophils, limited bactericidal activity, cytotoxic to many parasites</td>
</tr>
</tbody>
</table>
2) Oxygen Independent Mechanisms
Substances within leukocyte granules

Proteases within azurophilic granules
  • eg cathepsin G
  • antimicrobial properties for Gram -ve & +ve bacteria & some fungi
Failures of Phagocytosis: Survival of Phagocytosed Microorganisms

Some microorganisms can survive, either due to their own specialized protective mechanisms or due to a disorder of the host’s defense system.

1. **Escape phagosome, grow in cytoplasm**
   - eg, *Listeria monocytogenes, Rickettsiae*

2. **Block lysosome fusing with phagosome**
   - eg *Toxoplasma gondii, Salmonella enterica*

3. **Survive within phagolysosome**
   - eg, *Coxiella burnetti*
   - eg some *Mycobacterium sp*

[Diagram showing the processes of escape, block fusion, and survival in phagolysosome]
Failures of Phagocytosis: Survival of Phagocytosed Microorganisms

Some microorganisms can survive, either due to their own specialized protective mechanisms or due to a disorder of the host’s defense system.

Chronic granulomatous disease of childhood

- neutrophils incapable of producing $O_2^*/H_2O_2$ during phagocytosis
- results in recurrent infections
Tissue Damage Resulting During Phagocytosis
(extracellular release of leukocytic products)

Four basic mechanisms
1) Lysosomal suicide
2) Regurgitation during feeding
3) Reverse endocytosis (frustrated phagocytosis)
4) Neutrophil extracellular traps (NET’s)

Important Tissue Toxins
- lysosomal enzymes
- oxygen-derived active metabolites
- products of arachidonic acid metabolism (PG’s & LT’s)
a) Lysosomal Suicide - Cytotoxic Release

- causes:
  - pathogens overwhelm leukocyte
  - leukocyte dies for any reason.
- enzymes are released into ECM → pus or caseous exudate
b) Regurgitation During Feeding - Feeding Frenzies

- phagosome does not close
- enzymes are released into ECM → pus or caseous exudate
c) Reverse endocytosis (frustrated phagocytosis)

- antigen is too large to be internalized → can’t form phagosome
- enzymes are released into ECM → pus or caseous exudate
d) NETs (neutrophil extracellular traps) & EETs

- Neutrophils undergo apoptosis to release NETs (chromatin & histones) → forms meshwork to entrap and limit spread of released enzymes.

- Eosinophils don’t need to undergo apoptosis to release EETs.
Termination of the acute inflammatory response

• If all goes well….
  ➢ mediators are degraded soon after release (short half lives & produced in short bursts while stimulus is present)
  ➢ switch to anti-inflammatory **lipoxins** from arachidonic acid
  ➢ production of anti-inflammatory cytokines (**TGF-β**)
  ➢ inhibit the production of TNF-alpha in macrophages

• Otherwise, a chronic inflammation will develop
Patience, the light at the end of the tunnel is near.