

Inflammation

Course	 Immunology
Date	@April 9, 2024
Type	Lecture
Status	Completed
Reading	<input type="checkbox"/>

Summary

- Inflammation rapidly recruits cells of the immune system to the site of infection or tissue damage
- Complement, adhesion molecules, chemoattractants, MC and neutrophils all play important roles in this process
- Acute inflammation is usually beneficial
- Acute inflammation is an integral part of a successful immune response
- Required for the recruitment of cells to the site of the infection/injury and for the activation of the adaptive immune response
- Inflammatory process must be tightly regulated to avoid immune pathology
- Chronic inflammation is associated with many diseases

Inflammation can be defined as a response of the immune system of an organism to:

1. Infections
2. Non-infectious types of injury such as physical or chemical injury or damaged cells.

Classical signs of inflammation are **swelling, heat, redness, pain** and **loss of function** at the site of an immune response.

- The swelling, heat and redness reflect increased blood flow at that site and extravasation from the circulation into the tissue.

- Pain may be induced directly – due to the action molecules produced by immune responses have on nerve cells – or indirectly – due to mechanical swelling of the normal tissue, which stimulates nerve cells.
- Loss of function reflects tissue damage caused by immune responses, which may arise as a result of 'toxins' produced by the immune response targeted at a microbial organism causing collateral damage to host cells.

Inflammation is a crucial part of the immune response to harmful stimuli, such as pathogens, damaged cells, or irritants.

- In response to these stimuli immune cells (macrophages and mast cells predominantly) release **cytokines**, **chemokines**, **complement fragments** and other mediators, which leads to vasodilation and cell recruitment.

The function of inflammation

- Eliminate the initial cause of cell injury
- Clear out necrotic cells and tissues damaged from the original insult and the inflammatory process
- Initiate tissue repair

Usually the microorganism is eliminated by the **acute inflammatory response** and this type of inflammation is of benefit to the host as it represents the immune system 'doing its job'.

Three phases of acute inflammation:

- Vascular
- Cellular
- Resolution

However, if the immune response fails to eliminate the microorganism, or if for some other reason the inflammation does not resolve, then **chronic inflammation** ensues which usually results in '**immunopathology**' – damage to the host caused by the immune system itself.

Inflammation is the physiological response to clear a perceived danger, but also the cause of disease.

In this way inflammatory responses tread a fine line between protecting and harming the host

Recognition

Pathogen associated molecular pattern (PAMP) or damage associated molecular pattern (DAMP) recognised by Pattern recognition receptors (PRR)

- Surface (TLR, scavenger receptors, lectins)
- Within endosomal compartments (some TLR)
- Soluble in the cytoplasm (NOD like receptors, NLRs)
- Soluble (complement proteins, LPS-binding protein (LBP), ficolins, collectins)

Acute inflammation is initiated by macrophages and mast cells.

- **Macrophages** produce a range of cytokine and chemokines (including IL-1, TNF- α and IL-8) in response to PAMPs or DAMPs.
 - These cytokines have both local (paracrine) and long-distance (endocrine) effects.
- **Mast cells** play a crucial role in increasing permeability of blood vessels.
 - Mast cells contain **granules** which contain various mediators, especially **histamine** (vasoactive amino acid).
 - Mast cells can become activated in different ways: in response to PAMPs, in response to complement components and through binding of antigen to IgE antibody.
 - Histamine induces dilation of post capillary venules, activation of local endothelium and increase in blood vessel permeability.
 - In addition, a variety of complement fragments (see below) have the ability to trigger mast cells or act as chemoattractant for neutrophils.
 - As a result, **neutrophils** accumulate at the site of insult and can phagocytose invading pathogens.
 - Neutrophils have a short half-life and are usually cleared by apoptosis.



Acute inflammation is characterised by influx of neutrophils but not mast cells and macrophages as they are tissue resident. Vasodilation, and gap between endothelial cells become wider.

Complement

It was known over a hundred years ago that a relatively heat-sensitive factor in plasma was able to 'complement' the antimicrobial activity of immune serum.

The complement system is now known not to be a single substance but to comprise ~30 different proteins, which act in an **enzymic amplification cascade** system.

Complement is a cascade system

The complement system involves a series of molecules, including **zymogens** (inactive precursor enzymes), which act in a cascade fashion to generate components actively involved in host defence mechanisms. It can be activated in 3 different ways:

- Classical pathway activated by antibody-antigen complexes
- Lectin pathway activated by microbial sugars
- Alternative pathway activated by microbial cell walls

Its name originates from the fact that it 'complements' (enhances) the ability of antibody to dispose of microorganisms.

In addition to the complement components themselves, there are inhibitors of complement which play an important role in regulating the system and preventing complement from damaging our own cells and tissues.

The three pathways of activation

The all-important cleavage of C3 can be initiated in three ways.

Classical pathway

Requires the presence of **antibody**: recognises an antigen-antibody complex (immunological complex)

1. Binding of C1q, C1r, and C1s to antibody that are attached to the pathogen, activating C1
2. Subsequent activation of C4 and C2 to form the **classical pathway C3 convertase** enzyme (**C4b2a**) that cleaves C3 into C3a and C3b.

→ Wherever antibody molecules are bound to, (e.g. a bacterium) there will now be C3b as well, and since phagocytic cells can recognise both C3b and

antibody molecules, the chances that the bacterium will end up inside the phagocyte are tremendously increased.

Lectin pathway

- Similar to the classical pathway but is antibody-independent and is triggered by microbial surface sugar recognition by **mannose-binding lectin (MBL)** and subsequent binding to MBL-associated serine proteases (MASP-1 and MASP-2).
- The lectin pathway and the classical pathway join when C4b2 is formed and

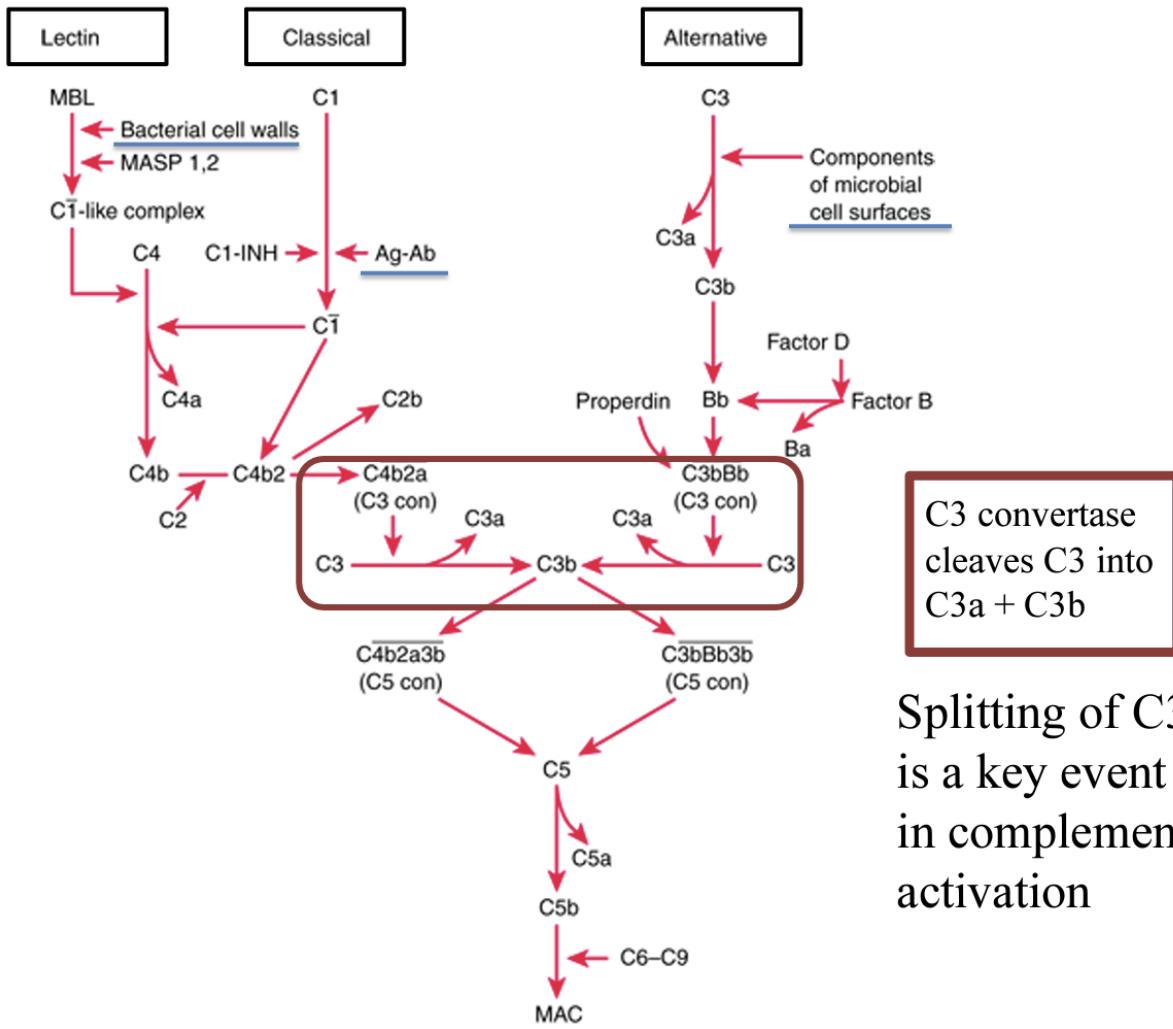
Alternative pathway

This is called the **alternative pathway** of complement activation because it was discovered after the first pathway to be found, which therefore became known as the 'classical' pathway.

- Always active in the background at a very low level when there is no activation
1. It starts with C3 binding to the cell surface such as bacteria with help from Factors B and D and **Properdin**
 - If bind to own cells there are inhibitory molecules
 2. The key enzyme that is produced is the **alternative pathway C3 convertase C3bBb** that converts C3 into C3a and C3b.

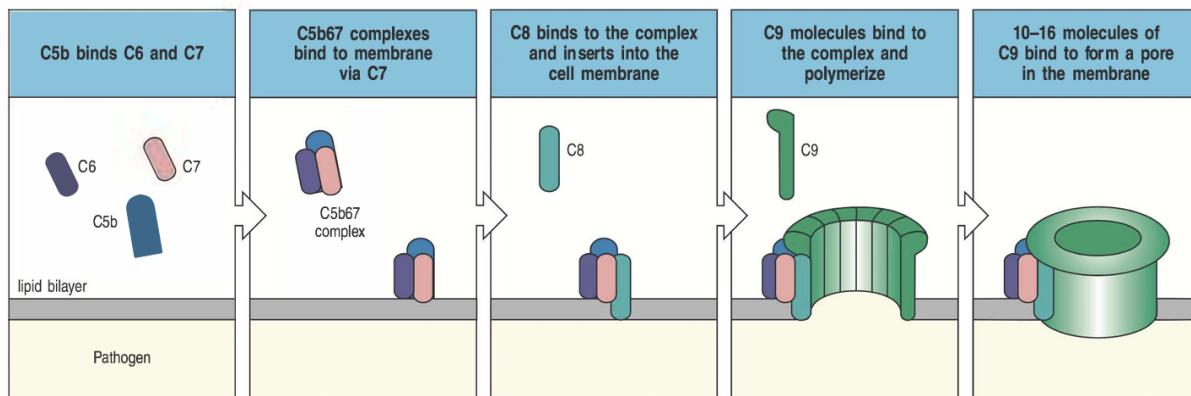
C3b are cause the generation of C5 convertase (C4b2a3b and C3bBb3b), which convert C5 to C5a and C5b

C5b binds to C6-C9, making a membrane attack complex (MAC)



- C3a, C3b, C5a are complement components involved in immune responses

Membrane attack complex (MAC)

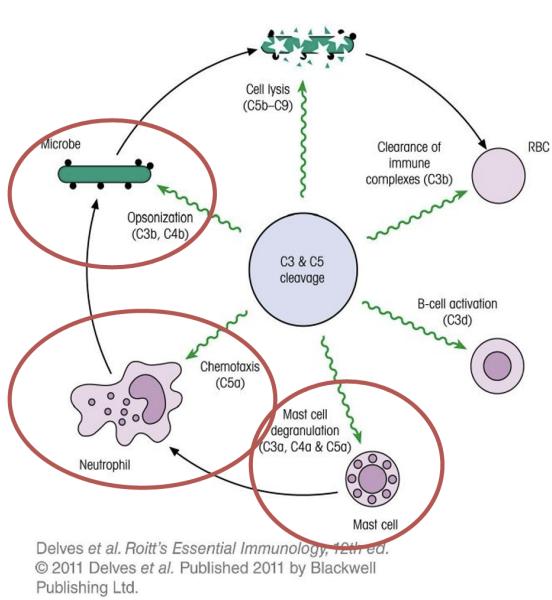


- A complement killing mechanism
- Polymerisation form a pore in the membrane to kill pathogens

- The ring structure is a pore in the membrane that allow free diffusion of molecules in and out of the cell
- If enough pores form, the cell is no longer able to survive
- This mechanism is very potent for some bacteria

Complements are always produced

- A certain level is in circulation
 - Liver always makes complement components such as C3 but not activated



- The activation of the cascade only occur once there is an infection
 - C3 and C5 are cleaved
 - C3a, C4a, C5a: mast cell degranulation
 - C5a: chemotoxins, similar to chemokines, important in neutrophil recruitment
 - C3b: opsonisation, coats the bacteria and phagocytes will recognise it via receptors - more effective phagocytosis

The combination of phagocytic cells, complement, and antibody represents a formidable obstacle to the survival of bacteria and fungi, and a deficiency of any one of these three elements can result in serious recurrent infections.

NB. Receptors for complement are found not only on phagocytes but also on erythrocytes (which thereby bind immune complexes for subsequent transfer to the spleen and liver where macrophages can destroy any bound pathogens) and on B lymphocytes (where complement receptors can act as costimulators and as inhibitory molecules).

Incidentally, the Epstein-Barr virus uses the complement receptor on B-cells to attach to these cells and infect them, causing glandular fever

There are inhibitors on the body's own cells to prevent the membrane-attack complex damaging them.

The central component: C3

The 3rd component, C3, is at the heart of the system.

- C3 is a major serum protein (about 1 mg/ml) whose enzymatic splitting leads to its activation.
- If the activation occurs on the surface of a bacterial cell, most of the C3 is left there in the form of the cleavage fragment C3b.
 - This marks the bacterium out for phagocytosis because phagocytic cells have receptors that recognise and attach to C3b.
 - The bacterium is said to be **opsonised** for phagocytosis.
- C3 also acts as a focus for the generation of C3a and, subsequently, C5a both of which cause mast cells to degranulate (C5a is also a neutrophil chemoattractant) and for C5b which joins with C6, C7, C8 and C9 to form the membrane-attack complex (MAC) which forms pores in the cell membrane leading to either cell lysis.

Opsonisation with **C3b** is a key event in marking many bacterial and fungal cells for subsequent destruction.

Lysis by the membrane attack complex is perhaps less crucial for protection against most organisms, but is important in protection against *Neisseria* species.

The other main cleavage fragment of C3, **C3a**, has a completely different function.

Together with **C5a** (derived by cleavage of C5), it promotes **inflammation** by activating mast cells, with the result that local vascular permeability increases and neutrophils, monocytes, antibodies, more complement flow into the tissues.

C5a also functions as a chemotactic factor for neutrophils, attracting them to the site of the infection.

Cytokines

- Acts as soluble messengers between cells (leucocytes) - coordinate
 - Interleukins (IL-1, IL-2)

- Interferons (IFN- α , IFN-gamma)
- Tumor necrosis factors (TNF α)
- Colony-stimulating factors
- Growth factors
- Chemokines (chemotactic cytokines)
- Pleiotropism (1 cytokine have multiple outcomes), Redundancy (different cytokines giving same outcome), Synergism, Antagonism (counteract the action of one another)
- Produced by activated immune cells and epithelium, stromal cells
 - Note that not only immune cells make cytokines
- Autocrine, paracrine, endocrine effects

Cytokines and Inflammation

- Induction phase
 - Pro-inflammatory cytokines: TNF-alpha, IL-6, IL-1 β
 - Local effects
 - Long range effects (liver, acute phase proteins, fever)
 - Interferons (IFN α and IFN β) induced by viral infection
 - Prevent viral replication, activate DC, macrophages, NK cell, induce chemokines
 - Both innate and adaptive immunity is activated
- Resolution phase
 - Anti-inflammatory cytokines, TGF β (repair), IL-10
 - Prevent over production and back to homeostasis

Chemokines

- Large family of small polypeptides (9-15 kDa)
- Denoted by L for ligand and a number e.g. CCL1,2,3... CXCL1,2,3
 - Nomenclature do with structure

Structural Signature				
<u>Class</u>			<u>Names</u>	<u>N</u>
CCC.....C.....C.....C.....		CCL#	24
CXCCX...C.....C.....C.....		CXCL#	17
CX3CCXXXC.....C.....C.....		CX3CL1	1
XCC.....C.....		XCL#	2

- More functionally descriptive names - e.g. monocyte chemotactic protein -1 (MCP-1)
- Chemokine receptors identified by numbers, e.g. CCR1, CCR2, CXCR1, CXCR2
- Redundancies - several chemokines display similar activities, shared receptor usage

Chemokines in Inflammation

- Inflammatory chemokines are only expressed during inflammatory episodes
- Main sources activated macrophages and DCs
- Cellular distribution of receptors dictates type of leukocytes recruited into tissues
 - Together with cellular adhesion molecules influence selective recruitment

Acute inflammation

Vascular phase

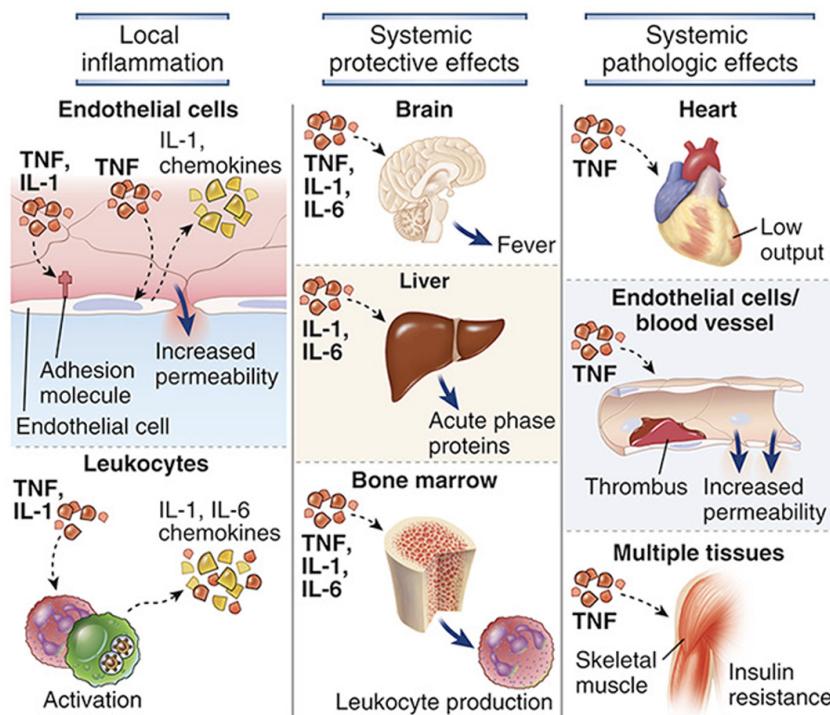
- Increased blood flow into infected tissues
 - More cells reaching the tissue
- Localised increase in vascular permeability
 - More space between cells to allow leucocytes, e.g. neutrophils, in
- Endothelium becomes adhesive ('activated')

Cellular phase

- Leukocytes accumulate in local vasculature
- Leukocytes migration into infected tissue

Removal of infectious agent

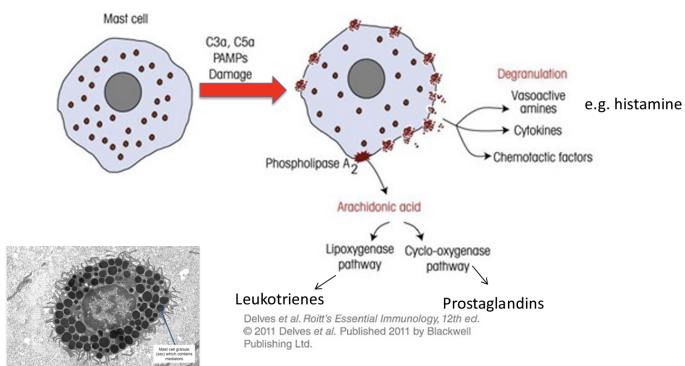
Resolution



- Liver makes more acute phase protein, to enhance the inflammation until it is no longer needed
- Bone marrow stimulated to make more leukocytes, due to more need to neutrophils to the site of infection

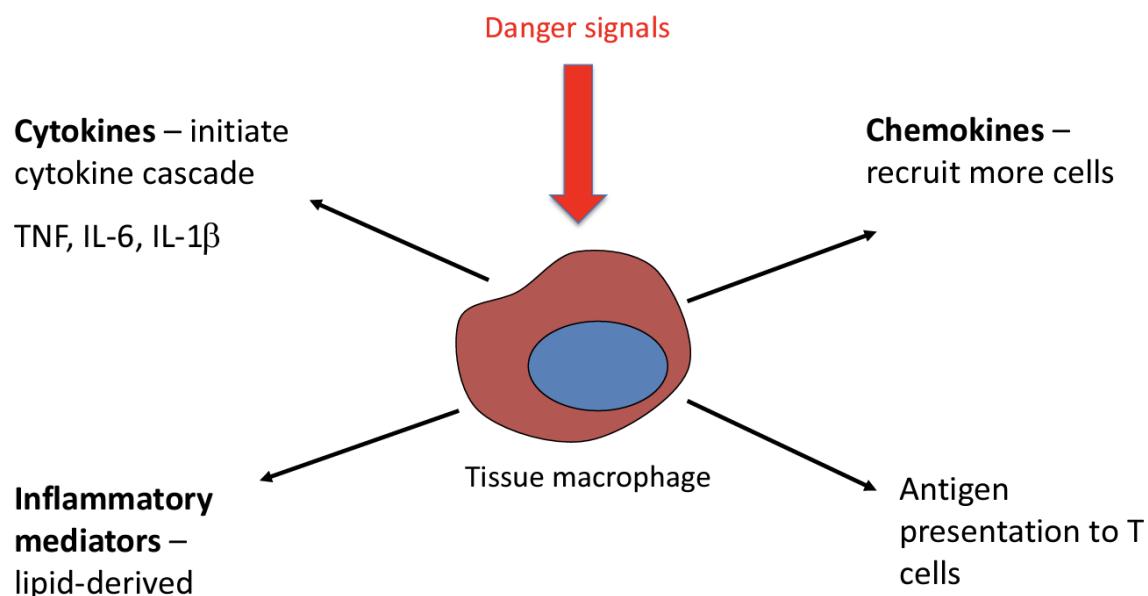
Inflammation involves mediators released from the tissue mast cells

- Mast cells full of preformed granules (e.g. histamine) degranulates when triggered
 - Trigger: C3a, C5a, PAMPs, Damage



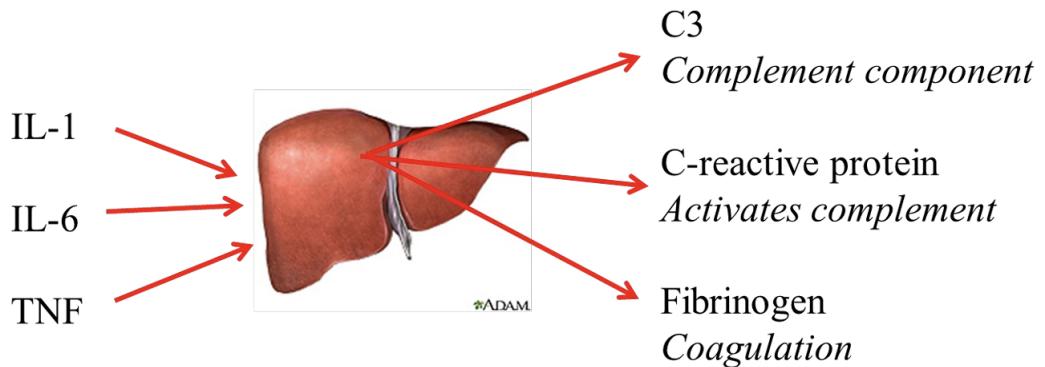
- Histamine causes vasodilation etc.
- Another pathway causing production of leukotrienes and prostaglandins
 - Impacts on endothelium to get more blood flow and more leaky endothelium

Macrophages play an important role in acute inflammation



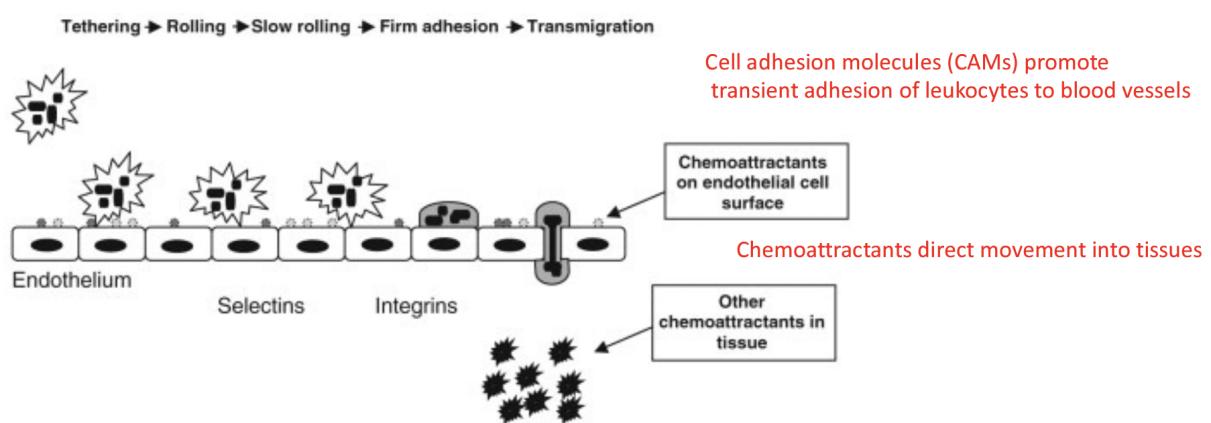
- Cytokines - initiate cytokine cascade
 - TNF, IL-6, IL-1 β
- Chemokines - recruit more cells
- Inflammatory mediators - lipid-derived
 - Prostaglandins and leukotrienes
- Antigen presentation to T cells

Acute phase response



- Enhances host resistance to infection and minimise tissue injury
 - A more distant action of different cytokines
- Promotes resolution and repair of inflammatory lesion
- Cytokines, released due to PRR activation, act on the liver to increase (up to 1000 fold) secretion of acute phase proteins (APPs)
 - C3: complement component
 - C-reactive protein (CRP): activates complement
 - Diagnosis of infection
 - Fibrinogen: coagulation
 - Isolation of infected area

Leukocyte migration



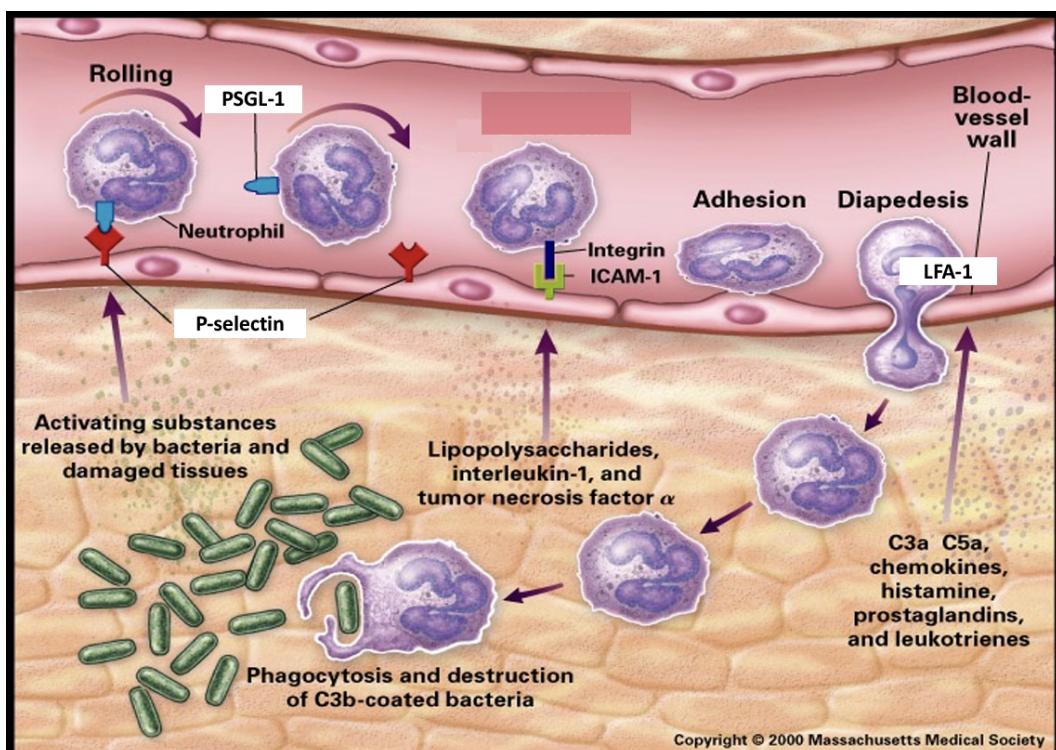
- Movement of leukocytes out of blood vessels (extravasation)

- **Highly ordered** process involving several different molecular events
 - The speed of the leukocytes circulate in the blood would not allow them to detect activation, needs to attach (tethering), rolling, attach, and cross the membrane (diapedesis)

Neutrophil

In the absence of an infection most neutrophils are present in a relatively inactive state in the blood circulation.

In order to accumulate at the site of **inflammation** circulating leukocytes need to leave the bloodstream and **enter the tissue**



Substances released from bacteria activate endothelium or MC and macrophages to make TNF

Tethering and Rolling

- In order to slow down, leukocytes need to adhere to the endothelial vessel.
 - This is achieved through interactions of **paired adhesion molecules expressed on leukocytes and on the vessel wall**
 - PSGL1 (on the neutrophils)- P selectin (vessel wall)
 - Rapidly expressed in response to **histamine**

- One of the first molecules upregulated on the blood vessel wall
- CD15 - E selectin
 - Following P selectin
 - Synthesized in response to **inflammatory cytokines**
- Lectin binds to sugar molecule (sLeX) on the neutrophil surface
- P selectin glycoprotein ligand-1 (**PSGL-1**) on neutrophils binds to **P-selectin and E-selectin** on the activated endothelium and this allows neutrophils to loosely attach and '**roll**' along the endothelium

Adhesion

- Interaction of **LFA-1** (integrin on the neutrophil) and **ICAM-1** (on the endothelium)
 - Neutrophils become activated and adhere strongly ('**arrest**') to endothelium
- These adhesion molecules (selectins and ICAM) become expressed **only upon infection** by the endothelial cells lining the blood vessels in response to:
 - Histamine, produced by mast cells
 - Later cytokines such as IL-1, TNF and IL-8, produced by macrophages

Diapedesis

- Neutrophils finally squeeze between endothelial cells into the tissue.
 - Vascular permeability increased by C3a, C5a, chemokines, histamine, prostaglandins and leukotrienes,
- PECAM1

Chemotaxis

- This is followed by the migration through the tissues under the influence of chemokines such as IL-8.
 - CXCR2 - CXCL8 (IL-8)

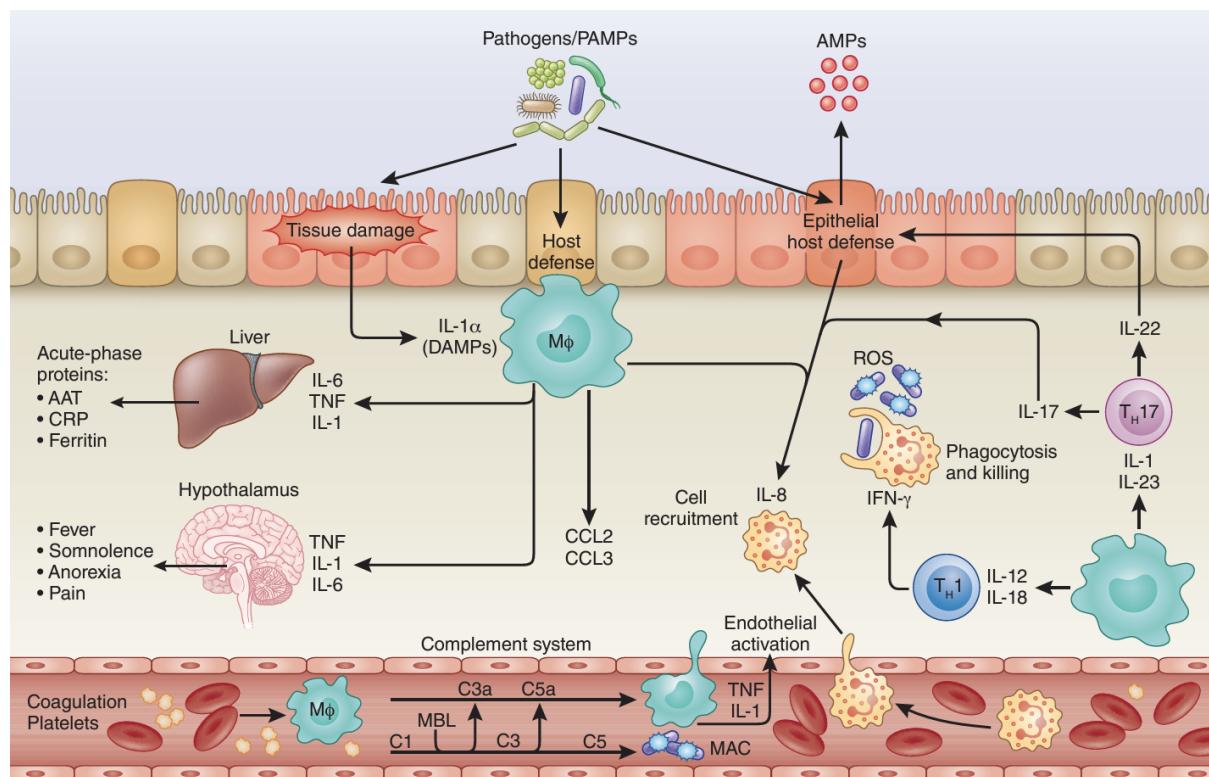
Ongoing inflammation

Tissue macrophages also contribute to inflammation, especially after the **very early phase** (2-4hrs after the initiation).

- Macrophages secrete cytokines such as **IL-1 β** and **TNF α**
 - Continue to activate the endothelium, inducing expression of E-selectin and sustaining the expression of P-selectin.
- Secrete **chemokines** which attract neutrophils (e.g. IL-8) and other types of leukocytes to the site of inflammation
 - Hopefully leading to the clearance of the invading microorganism.

Early immune response to infection: communication and cooperation

- Pathogen-caused tissue damage leads to DAMPs or PAMPs activate host defense (PRR)
- Macrophages release pro-inflammatory mediators to cause systemic effects in the liver and the brain as well as bone marrow
- Cell recruitment and expression of adhesion molecules on the endothelium
- Complement system also activated to increase vascular permeability allow cell migration



Prolonged high level of systemic inflammation: pathology

- Severe (bacterial) infection:
 - Strong / widespread TLR signaling
 - High levels of TNF α

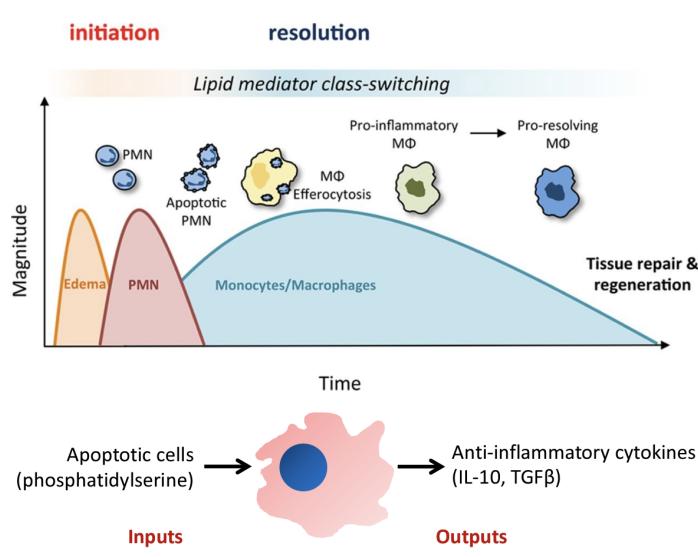
→ Sepsis

- Fast heart and respiratory rate
- Fever
- Metabolic abnormalities

→ Septic shock (little blood clots)

- Vascular collapse
- Disseminated intravascular coagulation

Resolution and chronic inflammation



- Clearance of infection
- Neutrophils undergo apoptosis
 - Apoptotic cells give signals expressing on the surface phosphatidylserine
- Causes their ingestion by macrophages, also changing macrophage phenotypes
 - Pro-resolving macrophages making TGF β , IL-10

- Neutrophils enter tissues and begin phagocytosis
 - After phagocytosis, become apoptotic
 - Even not, die within 3-5 days
- Monocytes follow some hours later (differentiate into macrophages)
 - At this stage it is pro-inflammatory producing TNF α
- Chemokines promote further recruitment

- Resolution

Once the agent that has provoked the inflammatory reaction has been cleared a number of regulatory processes take place which return the tissue to its 'normal' state - **homeostasis**.

Prostaglandin E (PGE2), Transforming growth factor beta (TGF-b**) and glucocorticoids** play an important role in regulation.

As a result, macrophages become deactivated, cytokine production is inhibited and cytotoxicity is reduced.

TGF β also plays an important role in **wound healing** repairing any tissue damage induced by inflammation/ immune response.

Alternatively, if the inflammation inducing insult persists (e.g. chronic infection or foreign body), the character of the inflammatory response changes.

- In the presence of a **persistent stimulus** prolonged neutrophil recruitment may lead to formation of an abscess and ultimately "**granulomatous**" tissue.
- The site of inflammation becomes dominated by **macrophages** and **lymphocytes** can also accumulate due to the contribution of the adaptive immune response.

Acute vs Chronic inflammation

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and lymphocytes (as the recruitment timeline)
Tissue, injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic signs	Prominent	Less prominent, may be subtle

Causes of chronic inflammation

- **Persistent** injury or infection - tuberculosis, ulcer, chronic viral infections
- **Prolonged** exposure to a toxic agent - pulmonary siliosis (silica in the lung)

- **Autoimmune disease** - self-perpetuating immune reaction that results in tissue damage and inflammation
 - Rheumatoid arthritis
 - Systemic lupus erythematosus
 - Multiple sclerosis



Constantly activate immune system

Chronic inflammation and disease

Chronic inflammation is also associated with numerous diseases (cancer, autoimmunity, arthritis, neurological disease) as well as ageing where it contributes to many different pathologies (atherosclerosis, cancer, Alzheimer's disease).

